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PITUITARY DWARFISM: DIAGNOSIS AND TREATMENT*

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ABSTRACT

Twenty-six cases of pituitary infantilism followed past the age of puberty were analyzed with regard to etiology, sex incidence, time of onset of growth retardation, and frequency of impairment of target-gland function, in an attempt to ascertain whether the diagnosis of pituitary insufficiency could have been established before the time of expected sexual maturation. Seven patients had neoplasms in the region of the sella turcica, whereas 19 had idiopathic pituitary insufficiency. There were 5 females and 2 males among the subjects with tumors, but only 1 female among the 19 patients with idiopathic hypopituitarism. The time at which growth retardation became manifest varied but tended to be after the age of 3, and showed itself earlier in the idiopathic cases than in the tumor cases. Studies of target-gland function indicated, apart from growth hormone and gonadotropin deficiency, impairment of thyroid and/or adrenocortical activity in 75 per cent of the cases as judged by serum cholesterol and protein-bound iodine levels, water diuresis, insulin sensitivity and 17-hydroxycorticoid excretion. It is therefore concluded that, when lack of either thyrotropin or adrenocorticotropin may be inferred from target-organ hypofunction in a dwarfed child, the diagnosis of hypopituitarism can be strongly suspected without awaiting failure of sexual maturation.

Psychologic evaluation indicated no dependent relationship between hypopituitarism and I.Q., but a dependent relationship to personality develop-

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ment—personality reflecting the problems concomitant with small stature. The management and available treatment of hypopituitary dwarfism is outlined and early substitution therapy with sex hormones is urged (though not before the usual age of puberty) once the diagnosis is reasonably certain.

THE determination of the cause of stunted growth is often most difficult during childhood. Bone disorders and systemic, metabolic or nutritional diseases should first be excluded. Hypothyroid dwarfism can be diagnosed on the basis of certain characteristic signs together with the study of the serum protein-bound iodine (PBI) and cholesterol. It then becomes necessary to decide whether short stature is due to, 1) merely a *constitutional delay in the rate of growth and development*, 2) *a "primordial" disturbance of growth*, or 3) true *pituitary deficiency*.

Contrary to popular opinion, pituitary deficiency is the least common cause of dwarfism, as shown in Table 1 (1). Out of 442 cases, only 30 (6.7 per cent) had hypopituitarism and 11 of these had tumors in the pituitary region. The largest group (probably 45 per cent) are children who lag 2 to 4 years behind the average in growth and development and have a late puberty, but eventually mature sexually and attain fairly normal adult heights.

Primordial dwarfs, comprising about 25 per cent of the group, are usually small at birth and are often mistaken for premature infants. Some have disproportions in the growth of the face and skull causing slender, pinched features and various other congenital anomalies. There is no significant lag in osseous development and they mature sexually at about the average time, unless they are born with primary defects in their gonads.

Patients with the "syndrome of gonadal aplasia and stunted growth"

TABLE 1. TYPES AND INCIDENCE OF STUNTED GROWTH

	No. of cases	Per cent
Constitutional retarded growth and adolescent development	130	29.4
Slow growth and retarded bone age (too young for diagnosis)	67	15.2
Primordial dwarfism 91		
Primordial with craniofacial anomalies ("progeroid") 23	114	25.8
Hypothyroidism (dwarfism conspicuous) (total hypothyroid subjects, 164)	53	12.0
"Syndrome of gonadal aplasia"	48	10.9
Hypopituitary or hypothalamic		
Pituitary deficiency 19		
Tumors 11	30	6.7
Total	*442*	*100.0*

(about 12 per cent) have also a primordial type of dwarfing but have no gonads. They appear to be girls and have female genital organs, but 80 per cent of them have a male chromatin pattern. Many of these patients have characteristic congenital anomalies such as webbed neck and coarctation of the aorta.

True hypopituitary dwarfs are usually normal in size at birth and the slowing of growth may not be apparent until they are 2 to 4 years of age. They are generally normal in appearance without congenital anomalies, but their features remain juvenile. The osseous development is usually markedly delayed by 5 or 6 years or more, secretion of pituitary gonadotropins is defective, and accordingly they remain permanently sexually infantile.

Before adult years the differentiation between pituitary deficiency and constitutional delay of growth and development is most difficult. Since there is no specific test for pituitary growth hormone, diagnosis depends upon demonstration of defects of the pituitary tropic hormones which regulate the functions of the thyroid, adrenals and gonads.

In the past we have refrained from making an absolute diagnosis of hypopituitary dwarfism until the patient had reached an age when the permanence of sexual infantilism was reasonably certain. The present paper consists of a survey of studies made on 26 patients, some of whom have been followed for twenty years or more, who have now reached an age at which there can be no reasonable doubt concerning the existence of hypopituitarism.

SELECTION OF CASES, PURPOSE OF SURVEY AND METHODS OF STUDY

The 26 selected patients all showed permanent sexual infantilism and considerable stunting of growth with a mean lag in height age of 5.8 years. There were 20 males and 6 females. Twenty-five of the 26 subjects were chosen from a total of 30 cases with pituitary infantilism past the age of puberty followed at the pediatric endocrine clinic of the Harriet Lane Home, the Johns Hopkins Hospital, 5 cases being rejected because of incomplete documentation or insufficient follow-up. The twenty-sixth subject, aged 43 years, was added from another clinic. Seven of the patients had neoplasms in the region of the pituitary and 19 had idiopathic pituitary deficiency.

The purpose of the survey was to determine the frequency of evidence of hypofunction of the target glands controlled by the pituitary in order to assess the value of various tests in establishing an earlier diagnosis of hypopituitarism. Some tests, notably glucose tolerance, insulin tolerance and serum cholesterol concentration, had been carried out years previously when the patients were children. Others, especially the serum PBI level, the "water load test," and the measurement of urinary 17-hydroxycorticosteroids were performed more recently for the purpose of this study. It is impossible to judge whether the present hypofunction of target glands could have been detected in earlier childhood.

In seeking clinical evidences of hypothyroidism in children with pituitary dwarfism one does not usually find sluggishness, impaired circulation, constipation and other characteristic signs. Accordingly, measurements of the serum PBI and cholesterol levels

and thyroidal uptake of I^{131} were used to detect the relatively moderate degrees of thyroid hypofunction. Similarly, definite clinical evidences of adrenal hypofunction such as sodium depletion, asthenia, hypoglycemia, hypotension and gastro-intestinal disturbances were usually absent, although 2 of the patients had hypoglycemic episodes in early childhood which disappeared later. Failure to respond to hypoglycemia induced by insulin has been considered as possible evidence of adrenal hypofunction (2). In the 1940's we carried out this test on many of our dwarfed children by administering insulin in a dosage of 0.1 or 0.05 unit/Kg. intravenously. Failure of the blood sugar level to rise to fasting values within 120 minutes was considered evidence of inability to respond to hypoglycemia. Because of the danger, as well as the difficulty of interpretation of the test, we have not employed it regularly in recent years.

It has been shown that prompt water diuresis is defective in Addison's disease and hypopituitarism (3). Therefore the ability to excrete a water load before and after a test dose of cortisone or ACTH has been suggested as a useful screening procedure for the detection of adrenal insufficiency (4, 5). Accordingly, we recently carried out this test in 15 of our hypopituitary subjects. After an overnight fast the patient was asked to empty his bladder and the urine was discarded. Tapwater (20 ml./Kg.) was administered by mouth over a period of fifteen to twenty minutes. Urine was collected hourly for four hours and the volume measured. Under these circumstances a normal person excretes 75 per cent or more of the water load, the largest quantity being excreted in the second hour. Subsequently the water loading test may be repeated after oral administration of cortisone (25 to 50 mg.) two hours prior to the ingestion of water, and the effect of the steroid administration observed. Decreased excretion of 17-hydroxycorticoids is probably the most definite evidence of diminished adrenal function. Unfortunately this measurement can be made only when proper laboratory facilities are available.

To assess pituitary gonadotropin activity, follicle-stimulating hormone (FSH) was estimated by the method of Bradbury, Brown and Brown (6) in some cases, and testicular biopsy specimens were obtained to determine testicular histology and the presence or absence of spermatogenesis.

RESULTS

Clinical findings

The clinical details of the 26 cases are summarized in Table 2. In 7, impairment of pituitary function was due to a tumor with expansion of the sella turcica, as shown on skull radiographs. The most common symptoms were headaches, nausea and vomiting followed within months or years by failing vision. One patient presented with sudden loss of vision in the left eye two years after having been seen for hypogonadism, whereas another requested medical consultation on account of stunted growth without other symptoms referable to a tumor. The onset of symptoms occurred between the ages of 4 and 12 years (mean 8.8 years) but months or years elapsed (mean 3.4 years) before medical advice was sought or the patient referred for special investigation. Most of the 7 patients were considerably stunted in height, and osseous development was delayed—being approximately equal to the height age. Dental development varied, the time of dental eruption being delayed in some but not in others.

TABLE 2. CLINICAL DATA

Subject and sex	Age at onset of symptoms or growth retardation (yrs.)	Age when first seen (yrs.)	Height (inches)	Height age (yrs.)	Bone age (yrs.)	Dental development*	Birth weight (lbs.)	Follow-up (yrs.)	Final height (inches)	Sexual development*	Comment
Tumors											
1. G.B. F	11	16.0	57.0	12.0	?	−		5	59.2	−	Diabetes insipidus
2. V.D. F	8	13.5	50.5	8.8	11	+		10	?	−	
3. C.G. F	4	8.8	39.0	3.8	4	+		5	—**	−	Hypernatremia
4. G.M. M	12	12.0	52.0	9.5	10	+		4	60.0	−	Diabetes insipidus
5. R.P. M	10	15.0	59.1	13.0	13	+		4	62	−	
6. B.S. F	5	21.0	53.5	10.0	15†	?		13	55	−	Diabetes insipidus
7. J.S. F	12	13.3	54.6	10.5	?	±		4	56.3	−	Diabetes insipidus
Idiopathic											
1. R.W.B. M	3	16.3	50.7	8.8	10	−	8.8	5	55.5	−	Panhypopituitarism
2. R.B. M	2	7.8	40.8	4.3	4	−	6.6	7	54.0	−	Panhypopituitarism
3. W.B. M	6	24.0	62.0	15.0	12	−	7.8	8	62.5	−	Panhypopituitarism
4. W.D. M	3	10.0	42.7	5.0	4	−	5.0	5	—**	−	Panhypopituitarism
5. J.F. M	5	43.0	61.0	14.0	14	±	?	1	61.5	−	Panhypopituitarism
6. A.H. M	4	17.0	51.0	9.0	11	−	8.0	12	61.1	−	Panhypopituitarism
7. J.H. M	3	5.5	36.0	2.8	4‡	−	6.8	28	57.4	−	Panhypopituitarism
8. R.H. M	?	5.2	35.0	2.5	3	−	9.0	16	55.5	−	Panhypopituitarism
9. H.P. M	6	11.5	48.0	7.5	8	−	5.0	3	—**	−	Panhypopituitarism
10. D.R. M	3	10.0	41.9	4.8	4	−	6.1	4	—**	−	Panhypopituitarism
11. E.S. M	1	6.6	30.0	1.3	3	−	5.8	9	46.8	−	Panhypopituitarism
12. A.T. F	4	8.5	42.5	5.0	4	−	7.9	29	59.2	−	Panhypopituitarism
13. J.W. M	?	10.0	40.5	4.0	4	±	7.0	10	?	−	Panhypopituitarism
14. M.W. M	8	14.0	51.5	9.0	9	−	8.0	10	62.5	−	Panhypopituitarism
15. H.J. M	?	9.0	40.8	4.5	5	+	5.0	7	—**	−	Partial hypopituitarism
16. H.P. M	1	15.3	52.0	9.5	12	+	6.3	7	?	−	Partial hypopituitarism
17. D.R. M	1	4.5	32.0	1.5	4§	+	4.8	18	48.0	−	Partial hypopituitarism
18. F.S. M	1	7.2	36.5	3.0	4	±	?	20	53.0	±	Matured sexually 23 yrs.
19. N.W. M	2	8.3	41.0	4.3	4	−	7.5	20	60.0	±	Matured sexually 20 yrs.

* + Normal.
− Delayed or absent.
** Still growing.
† After some treatment.
‡ At age 9 years.
§ Bone age 12 at age 16.

In the remaining 19 patients the cause of the pituitary insufficiency was unknown. The pattern of impairment of growth and development in this group of cases with idiopathic hypopituitarism, however, was identical with that in the tumor group, except that the onset of growth retardation occurred at an earlier age. Signs and symptoms referable to a tumor were absent. Failure to grow normally was first noticed at 1 to 4 years of age, though in some not until they entered school at the age of 6 or more. The

age at which the patients were first seen ranged from 4½ to 43 years. Excluding the patient aged 43, the mean age when medical advice was sought on account of shortness of stature was 9.8 years—somewhat less than the age in the tumor group. The 43-year-old subject had a height age of 14 years and measured 61 inches in height. In the rest of the subjects the height varied from 30 to 62 inches, indicating a general lag in height age of 4 years or more. In 17 of the 19 cases the bone age and osseous development were strikingly retarded and in keeping with the patient's height age. In 1 case the bone age was between the height age and the chronologic age, whereas in the last case the bone age was in keeping with the chronologic age at 4 years but was four years retarded at the age of 16 years.

In 2 of the patients, F.S. and N.W., the testes were noticed to have enlarged spontaneously after some years of testosterone substitution therapy, at the ages of 20 and 23 respectively. In both cases the testes reached adult proportions and spermatogenesis, albeit reduced, was demonstrated. However, neither patient became completely virilized, facial and body hair remained practically absent, and the facial appearance maintained its youthful look, to which wrinkling was later added. The ultimate height reached by these 2 subjects was only 53 and 60 inches, respectively, so that stunting remained a noticeable feature.

Among the 7 patients with pituitary tumors there were 5 females and 2 males. Of the 19 patients with idiopathic hypopituitarism only 1 was a female and 18 were males.

Laboratory findings

The laboratory findings in the 26 patients are summarized in Table 3. All the 7 tumor patients with pituitary infantilism showed, in addition to deficiency of growth hormone, evidence of depressed function of target organs due to deficiency of pituitary tropic hormones. Six of the 7 patients had lowered thyroid function, as judged by serum cholesterol values above 300 mg. per 100 ml., and 3 of these had PBI levels below 4.0 μg. per 100 ml. Of the 4 patients tested, 2 manifested increased insulin sensitivity and hypoglycemic unresponsiveness. Prompt water diuresis was lacking in 1 of 2 patients given a water load. Urinary 17-ketosteroid excretion was abnormally low in the 2 patients in whom it was measured. 17-Hydroxycorticosteroid excretion was normal in the 2 subjects studied, although 1 of them was being maintained with oral cortisone, 10 mg. daily. Urinary gonadotropins were not measurable in the 2 cases in whom they were assayed.

Failure of either thyrotropic, adrenocorticotropic or gonadotropic hormone production or any combination thereof in addition to growth hormone deficiency, could similarly be inferred in 14 of the 19 cases with idio-

TABLE 3. LABORATORY DATA

Subject	Thyroid		Adrenal				Pituitary
	Serum cholesterol (mg./100 ml.)	Serum PBI (μg./100 ml.)	Urinary 17-KS* (mg./24 hrs.)	Urinary 17-OH-CS* (mg./24 hrs.)	Insulin tolerance**	H_2O load (% excreted)	Urinary FSH (m.u./24 hrs.)
Tumor							
1. G.B.	357	5.5					
2. V.D.	300	5.5			A		<6.6
3. C.G.	474	2.3	1.0	N	N	100	
4. G.M.	298	1.9	1.4			10	<6.6
5. R.P.	334	3.8					
6. B.S.	313	4.1			N		
7. J.S.	370	4.2		2.1†	A		
Idiopathic							
1. R.W.B.	140		1.5		A		<6.6
2. R.B.	370	1:9	1.3		N	48	
3. W.B.	380	2.4	1.1	0.1	N		
4. W.D.	344	4.4			A		
5. J.F.	200‡	4.0	.9	0	N	20	<6.6
6. A.H.	564						
7. J.H.	334	4.0			A	22	
8. R.H.	380	4.2	1.2	0.6	A	50	<6.6
9. H.P.	356	2.9	1.4	0.7	N	31	
10. D.R.	340§	3.5		0.3	A	64	
11. E.S.	354	3.5	1.1		A		
12. A.T.	323				A		
13. J.W.	400	4.5	2.2	0.1	A	15	
14. M.W.	326	3.3	1.5	0.4	A	18	<6.6
15. H.J.	310	5.3				106	
16. H.P.	224	4.6	5.5	3.4	N	105	
17. D.R.	234	6.0	2.2	4.5	N	95	
18. F.S.	160	4.5	3.8	13.1¶	N	88	<6.6
19. N.W.	335	5.0	2.8	5.4	N	81	>6.6<13.2

* Measured after the age of 14 years before commencing androgen therapy.

** A = abnormal.
N = normal.

† While taking oral cortisone 10 mg. daily.

‡ B.M.R. −31 per cent; thyroidal I^{131} uptake 12 per cent.

§ Thyroidal I^{131} uptake 8 per cent.

¶ Plasma level in μg.=/100 ml.

pathic hypopituitary dwarfism, in view of the low level of function of the respective target organs. The concentration of serum PBI was estimated in 16 subjects, and in 8 it was found to be 4.0 μg. per 100 ml. or less. The

serum cholesterol level was raised above 300 mg. per 100 ml. in all but 4 patients; by itself, this finding was not accepted as evidence of thyrotropic hormone failure. In none was the serum cholesterol level as high as in untreated cases of primary hypothyroidism, nor did any of the subjects exhibit clinical symptoms or signs of frank thyroid insufficiency. The radioactive iodine (I^{131}) uptake by the thyroid gland was studied in 2 cases. In both, it was at the lower limit of normal or just below, and was markedly stimulated by administration of thyrotropic hormone.

Increased insulin sensitivity and hypoglycemic unresponsiveness were present in 9 of 17 patients tested. Excretion of a standard water load was studied in 13 cases and found to be abnormal in 8. Urinary 17-ketosteroid excretion was abnormally low in 12 of 13 patients in whom it was measured, and 17-hydroxycorticosteroid excretion was similarly affected in 7 out of 11 patients studied. Urinary follicle-stimulating hormone was not measurable at puberty in 5 of 6 cases in whom it was assayed. Testicular biopsy specimens obtained in 3 cases (W.B., J.F. and N.W. at the age of 18 years) showed infantile tubules with absence of maturation and spermatogenesis and no Leydig cells.

From the laboratory data it may be concluded that 14 of the 19 patients with hypopituitary dwarfism showed, in addition to lack of growth hormone, definite evidence not only of a deficiency of follicle-stimulating hormone but also of thyrotropic and/or adrenocorticotropic hormones, as judged by target-organ function. In fact, 12 subjects showed evidence of impairment of function of both the thyroid and the adrenal glands. In 5 patients there was no evidence of impairment of thyroid or adrenal function, except for low urinary 17-ketosteroid excretion and an elevated serum cholesterol level in 2 of them. The concentration of serum protein-bound iodine, insulin sensitivity, water diuresis and urinary 17-hydroxycorticosteroid excretion were normal. Two of these patients matured sexually to some degree and in 1 urinary follicle-stimulating hormone could be measured.

Comment of findings

The incidence of positive laboratory findings corroborating the diagnosis of pituitary infantilism is shown in Table 4. As expected on the basis on which the cases were selected, the tests most frequently yielding abnormal results were the estimations of 17-ketosteroid and follicle-stimulating hormone excretion. Since neither of these tests is of diagnostic value until the age of puberty, they cannot be used in establishing the diagnosis during childhood. They are therefore omitted from further discussion.

Most cases of pituitary tumor present with symptoms and signs of increased intracranial pressure but with few if any endocrine disturbances,

and are rarely referred to an endocrine clinic. It is therefore not surprising that those patients with pituitary destruction who show stunting of growth should also show other tropic hormone deficiencies. In the present series, in all 7 cases of pituitary infantilism due to destructive lesions there was evidence of failure not only of the growth hormone but also of either the thyrotropic or adrenocorticotropic hormone. However since the diagnosis is based on skull radiographic findings and impairment of visual fields rather than on the results of laboratory tests, the recognition of hormonal deficiencies is of importance only as far as treatment is concerned.

In the panhypopituitary group of patients the pattern of hormonal deficiencies closely resembled that in the tumor group. The test most easily

TABLE 4. THE INCIDENCE OF POSITIVE LABORATORY FINDINGS

Type of case	No. of cases	*Thyroid hypofunction*		*Adrenal hypofunction*				*Gonadotropi hypofunction*
		Serum cholest., >300 mg./100 ml.	Serum PBI, <4 μg./100 ml.	No response to hypoglycemia	Excretion of H_2O load <65%	Urinary 17-OH-CS <2 mg./24 hrs.	Urinary 17-KS <3 mg./24 hrs.	Urinary FSH absent
Organic	7	6 (7)*	3 (5)	2 (4)	1 (2)	1 (2)	2 (2)	2 (2)
Idiopathic (panhypopit.)	14	12 (14)	8 (11)	9 (13)	8 (8)	7 (7)	9 (9)	4 (4)
Idiopathic (partial)	5	2 (5)	0 (5)	0 (4)	0 (5)	0 (4)	3 (4)	1 (2)
Total	26	20 (26)	11 (21)	11 (21)	9 (15)	8 (13)	14 (15)	7 (8)

* Figures in parentheses indicate the total number of tests in each group.

carried out and of the greatest diagnostic value was that for water load excretion. This test seems to reflect closely the functional integrity of the pituitary-adrenocortical mechanism, provided disturbances of absorption and hepatic, cardiac or renal disease are absent. In all the cases in which the test yielded abnormal results, 17-hydroxycorticosteroid excretion was subnormal. Similarly the water load test yielded abnormal results in all patients with increased insulin sensitivity and hypoglycemic unresponsiveness who were subjected to both tests. Since insulin sensitivity was normal in some instances in which water excretion was abnormal, it may be assumed that the latter is a more sensitive index as well as a less dangerous test for adequacy of adrenocortical function, provided the serum sodium level is normal. Measurement of urinary 17-hydroxycorticosteroid excretion remains the most satisfactory test for adrenocortical function, and should be carried out. The water test is valuable as a screening procedure.

The serum protein-bound iodine level was low in all cases of the panhypopituitary group, although in only 8 of the 11 cases tested was it below

the lower limit of normal. The serum cholesterol level was above 300 mg. per 100 ml. in 12 out of 14 patients examined (though not consistently); it tended to vary without relation to food intake or other obvious factors and bore no relation to the protein-bound iodine level; abnormally high values were obtained at one examination and normal ones at another. The same tendency to fluctuation is also seen in hypothyroidism, though at a higher level. The thyroidal I^{131} uptake and the response of the thyroid to exogenous thyrotropic hormone offer another useful diagnostic test.

In 5 of the 19 subjects with idiopathic hypopituitarism, tests for thyroid and adrenocortical function failed to show any gross evidence of deficiency of tropic hormones. In 2 cases hypercholesterolemia was present at times, and in all 5 cases 17-ketosteroid excretion was low at puberty. In 1 case the excretion of follicle-stimulating hormone was at the lower limit of the normal range. Nevertheless, since stunted growth and sexual infantilism were noticeable features, the diagnosis of pituitary dwarfism seems justified.

Whether the delayed sexual maturation in Patients F.S. and N.W. was the result of eventual activation of the pituitary-gonadal mechanism or the result of prolonged testosterone substitution therapy remains a matter of conjecture. Similar results have been reported previously without any definite conclusions having been reached (7, 8).

The marked preponderance of males over females in our group of patients with idiopathic hypopituitarism is worthy of note, even though the number of cases is small. The significance is at present obscure.

In the present series of subjects with dwarfism of pituitary origin, 75 per cent exhibited evidence of either thyroid or adrenocortical hypofunction. Although this is a small series, the findings suggest that the diagnosis can probably be established before puberty in a significant proportion of cases.

TREATMENT

Although growth hormone prepared from animal pituitaries has proved to be highly active in rats, it has not been shown to accelerate growth or protein anabolism in man. Among our 26 patients there were 6 who, over a number of years, received various growth fractions, pure growth hormone prepared by the method of Russel and Wilhelmi, and other preparations claimed to be growth-promoting, without discernible effect.

Thirteen patients were treated with thyroid in view of laboratory evidence of depressed thyroid function. Although the lowered level of serum protein-bound iodine and the raised level of cholesterol returned to normal during thyroid therapy, there was no indication either subjectively or objectively of any marked change in the clinical condition. The administration of thyroid had no effect on osseous development nor did it hasten

epiphyseal maturation in our cases. In adults with severe hypopituitarism accompanied by secondary hypoadrenalism and hypothyroidism, the administration of thyroid hormone may precipitate an adrenal crisis. Although our patients tolerated thyroid in relatively large doses without cortisone supplementation, it may be safer to institute cortisone therapy before beginning thyroid.

Four subjects with low excretion of urinary 17-hydroxycorticosteroids and abnormal excretion of a water load were given in addition to thyroid medication a trial treatment with oral cortisone, 25 mg. daily in fractional doses. Although there was improvement in water excretion, there was otherwise little objective evidence of a beneficial effect. However 2 of the 4 patients considered themselves markedly better with regard to energy. stamina, and drive; they felt less tired and more alert. The other 2 subjects were uncertain of the benefit derived from the cortisone. In 2 additional cases due to tumors, treatment with cortisone unmasked latent diabetes insipidus due to posterior hypophyseal insufficiency.

Three patients were given chorionic gonadotropin in dosages of 500 to 2000 R.U. three times a week for up to nine months. Although this led to some degree of testicular maturation and a rise in urinary 17-ketosteroid excretion with concomitant signs of virilization, there was immediate regression once treatment was stopped.

In the cases in which it was indicated, Pitressin was used for the control of diabetes insipidus.

Sex hormone substitution therapy was given to all our patients once it became obvious that spontaneous puberty was not to be expected.

Males

Most boys were given testosterone, either as long-acting testosterone cyclopentylpropionate or testosterone enanthate, once every three to four weeks in doses of 200 mg. to 400 mg. A few were treated with oral methyltestosterone linguets, 30 mg. daily, but this form was thought to be somewhat less efficacious.

Androgen therapy led in all patients to a growth spurt with gain in weight, marked broadening of the frame, and muscular development. The voice became lower, the penis and prostate gland enlarged and there was some growth of pubic hair, usually discernible within the first six to eight weeks of treatment. Spontaneous erections occurred in most cases, more commonly in the initial stages of treatment than later. During treatment with depot testosterone, erections occurred more frequently during the first two weeks following the injection and decreased in frequency as the effect of the hormone wore off. In some cases nocturnal emissions occurred but masturbation was neither common nor frequently practiced in this

group of patients. Sexual phantasies were reported but it was extremely rare for any of the subjects to have an active erotic interest in the opposite sex.

The degree of virilization achieved in these patients was considerably below normal. In marked contrast to patients with primary testicular hypogonadism, there was little growth of facial, pubic or axillary hair and the prostate remained below the average adult size. There was aging of the facial appearance during androgen therapy, but even then the patients retained their somewhat rounded faces and immature look.

Growth was most rapid in the first six months or year, amounting to as much as 5 inches. The speed of growth then diminished over the next two or three years, until growth finally ceased due to closure of the epiphyseal lines. None of the subjects reached a height in excess of 62½ inches and only 6 of the 26 reached a height of 60 inches or more. The patient who had remained untreated until the age of 43 years had a height of 61 inches and it is very questionable whether the ultimate height attained by our treated patients was greater than might have been reached eventually without treatment.

One of the subjects included in the present report (in addition to others not included) was treated with 19-nortestosterone to assess whether the action of this compound was protein anabolic and non-virilizing. Careful study over many months showed that the substance is biologically weakly androgenic. The impression was gained that there was not sufficient dissociation between growth-promoting and androgenic effect to make the drug clinically useful.

Psychologically, androgen therapy, in causing growth, muscular development, improved stamina and some virilization, proved beneficial to these subjects. It increased their assurance and aggressiveness, and enhanced their ability to cope with the difficulties of every-day life. The success in psychologic adjustment, however, was not matched by social participation. Practically all the group remained somewhat shy and withdrawn. They lacked confidence in their own manhood and avoided dating and petting, despite the fact that physically they were sufficiently well developed to have managed sexual intercourse successfully.

Since smallness of stature alone does not explain this failure of sociosexual maturation—even though it is generally conceded that testosterone ordinarily increases libido—it is our belief that the lack of confidence in our patients may to some extent be related to the late institution of sex hormone replacement therapy. Most of our subjects were not treated before the age of 15 or later.

Females

Since administration of estrogens alone does not cause acceleration of growth, nor growth of pubic hair, and in fact may hasten epiphyseal fusion, the female patients were treated with androgens as well as estrogens. The administration of methyltestosterone (10 to 20 mg. daily) in addition to stilbestrol (1 mg. daily) resulted in a near-normal growth of pubic hair and a spurt of growth without producing virilization. Testosterone therapy had to be continued, as otherwise loss of body hair would be expected. After six to nine months of combined androgen and estrogen therapy, when development of the breasts, nipples, labia and vagina, estrinization of the vagina, and growth of the uterus had occurred, periodic therapy was instituted unless bleeding had taken place previously.

With this treatment, physical maturation was more complete than in the case of the males. The improvement in morale and the social and psychologic adjustment achieved by these female patients appeared, on the whole, to be better than that of their male counterparts. One of the patients has married, says she has satisfactory sexual intercourse, and lives a happy and contented life although she and her husband understand that fertility is probably impossible.

Psychologic considerations

Twelve of the 26 patients were given a Wechsler intelligence test. The intelligence quotients[1] ranged from 64 to 123, their scatter conforming fairly closely to normal curve distribution. Evidently there was no dependent relationship between pituitary insufficiency and I.Q.

By contrast, a dependent relationship between pituitary insufficiency and personality development was evident. Personality always reflected the problems concomitant with small stature, even though there was no uniform personality disposition shared by all subjects. Being small and of graceful appearance these children were invariably pampered and overprotected—it is unbelievably difficult for adults not to treat a dwarfed child as a cute mascotish baby doll—with the result that they responded with a strong tendency to act and talk babyishly, in conformity with their size and not their years. As they advanced far into the teen-age without sex hormone substitution therapy they became progressively more seriously retarded in their psychologic and social development and adjustment. Although often considered happy and serene people untroubled by the emotional upheavals of adolescence, they were in the position of "the boy

[1] The testing for IQ was performed by Dr. John Money, to whom thanks are also due for some of the ideas embodied in the next paragraph.

who never grew up" but was not content because his failure to mature was not of his own choosing. Eventually some reached the point where they became permanently fixed in psychologic juvenility and were unable to utilize the advantages of induced physical maturation in order to progress to full psychologic adulthood.

GENERAL COMMENT

The diagnosis of pituitary insufficiency in subjects with stunted growth and developmental retardation due to the presence of a demonstrable lesion in the hypothalamic-pituitary region presents no difficulty. The degree to which pituitary function may be impaired is variable and there are many cases in the literature of pituitary tumors wtih minimal or no endocrine disturbances.

The main difficulty in the diagnosis of idiopathic pituitary dwarfism is the absence of a rapid and reliable method for measuring physiologic levels of growth hormone in biologic material. Hence in the absence of obvious pathology the diagnosis is at best based on indirect evidence. However, the close similarity of both clinical and laboratory findings in the patients with tumors and in the idiopathic hypopituitary group is sufficiently striking and strongly suggestive of the same functional abnormality.

It is recognized that hypopituitarism may involve all the pituitary hormones or may be limited to failure of secretion of only 1 or 2 hormones. Since failure of gonadotropin alone does not lead to stunting of growth, but on the contrary to excessive growth in length, the absence of sexual maturation in a dwarfed subject is strong evidence for a pituitary disorder.

When lack of either thyrotropin or adrenocorticotropin can be demonstrated, as manifested by abnormal target-organ function in a dwarfed child, the diagnosis can be strongly suspected without awaiting failure of sexual maturation. In those cases in which the deficiency is confined to growth hormone and gonadotropin, sexual maturation or lack thereof has to be awaited before the diagnosis can be established.

As regards treatment, we feel that administration of thyroid and cortisone is of no benefit in the absence of conclusive evidence that these hormones are deficient. Even when the laboratory data indicate their use, the results, though subjectively encouraging, are objectively disappointing. Treatment with the sex hormones has a very definite place in the management of the hypopituitary child, *though not before the usual age of puberty.* Although the administration of chorionic gonadotropin would appear to be the logical approach, there is some evidence, perhaps not yet conclusive, that its prolonged use may lead to permanent damage to the gonads (Wilkins, unpublished data).

In the absence of an effective growth hormone preparation, the treat-

ment of choice appears to be substitution therapy with testosterone and estrogen, since these steroids cause a growth spurt with concomitant weight gain and virilization or feminization. This treatment should be instituted at about 12 to 13 years of age, once the diagnosis of hypopituitarism has been firmly established and there is reasonable certainty that the case is not one of constitutionally delayed growth and adolescence. By relatively early treatment these children may be saved considerable psychologic difficulty and may be helped greatly in their social adjustment. The ultimate height reached by these dwarfs may not be materially altered by the form of therapy available at the present time, but this does not by any means mitigate against its use.

There are obviously many facets of pituitary hypofunction which have so far eluded understanding, and clarification and further work are indicated. Nevertheless, it is hoped that the present report will help the clinician to recognize and diagnose the condition and institute available treatment.

Addendum

Since this paper was submitted for publication, our attention has been drawn to an article by J. R. Bierich (Über den hypophysären Zwergwuchs im Kindesalter, *Medizinische* No. 38: 1375 (Sept. 21) 1957) in which 15 cases of hypopituitarism were investigated. The findings and conclusions were almost identical with our own.

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ABSENCE OF 21-DEHYDROXYLATION IN CONGENITAL ADRENAL HYPERPLASIA*

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ABSTRACT

Hydrocortisone-4-C^{14} was administered intravenously to a patient with congenital adrenal hyperplasia who excreted large amounts of C-21 deoxyhydrocortisone metabolites in his urine. By the reverse isotope dilution technique, it was found that 3α,17α-dihydroxypregnane-11,20-dione, 11-ketopregnane-3α, 17α,20α-triol and pregnane-3α,11β,17α,20α-tetrol accounted for less than 0.3 per cent of the radioactivity in the neutral steroid extract of the 24-hour urine following administration. Hydrocortisone-4-C^{14} administered to a leukemic patient yielded no radioactivity in pregnane-3α,11β,17α,20α-tetrol and 11-ketopregnane-3α,17α,20α-triol added to the neutral steroid extract of the urine during the fourth through sixth hour inclusive following administration. It is concluded that the large amounts of C-21 deoxysteroids present in the urine of patients with congenital adrenal hyperplasia do not arise from hydrocortisone or other C-21 oxygenated hormones. The precursors are C-21 deoxysteroid hormones.

CONGENITAL adrenal hyperplasia is characterized by the inability of the adrenal gland to complete the synthesis of hydrocortisone and, as implied by the name, is a genetically determined "inborn error of metabolism" (1). In a more strictly biochemical sense the lesion has been defined within either of 2 classifications: 1) a relative deficiency in the introduction of oxygen at C-21 in a 21-carbon substrate, or 2) failure of 11-oxygenation with both 21-carbon and 19-carbon substrates (2–4). Other related chemical defects can be foreseen but none has as yet been subjected to experimental analysis. Although congenital adrenal hyperplasia appears to be chemically defined, the possibility existed that the same pathologic condition could result from a change in the peripheral metabolism of a normal hormone. Specifically it could be postulated that, for some reason, removal of the C-21 hydroxyl group of hydrocortisone became a prominent feature in the metabolic transformation of that hormone in congenital hyperplasia. As a consequence many tissues would be exposed to this altered hormone rather than to the compound secreted by the adrenal. Among other effects, the suppression of pituitary secretion by this 21-deoxysteroid would be inefficient by comparison with hydrocortisone, and thus more adrenocorticotropic hormone (ACTH) would be produced. The adrenal, in turn, under stimulation would elaborate more hydrocortisone

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and the cycle would be established. This explanation would account for the presence of considerable quantities of C-21 deoxysteroid metabolites in the urine (3–5) and would be consistent with the finding of high blood levels of ACTH (6). This study was undertaken to explore this possibility.

SUBJECTS AND METHODS

Subject R was a 36-year-old man with congenital adrenal hyperplasia. Approximately 1 mg. of hydrocortisone-4-C^{14} (4.77×10^6 c.p.m.)[1,2] in 165 ml. of 5 per cent glucose and 2 ml. of ethanol was administered intravenously over a period of thirty minutes. The urinary steroid excretion of this patient has been recently reported (4).

The urine specimens obtained during the first twenty-four hours following the infusion of hydrocortisone-4-C^{14} were combined (4.29×10^6 c.p.m.). After addition of a small amount of Versene, the urine was incubated with 300 Fishman units of Ketodase[3] (beef liver β-glucuronidase) per ml. at 37° and pH 5.0 for five days. At the end of incubation, after adjustment to pH 1, the urine was continuously extracted with ether for forty-eight hours. The neutral fraction, obtained after extraction of the ether solution with aqueous base and with water, contained 2.77×10^6 c.p.m. To this extract the following pure steroids were added: 34.7 mg. of 11-ketopregnane-3α,17α,20α-triol; 35.9 mg. of pregnane-3α,11β,17α,20α-tetrol; and 36.5 mg. of 3α,17α-dihydroxypregnane-11,20-dione. The extract was chromatographed on 80 Gm. of silica gel containing 32 ml. of ethanol and eluted with increasing amounts of ethanol in chloroform at a rate of 10 ml. per hour.

Elution with 3 per cent ethanol in chloroform yielded fractions containing 25 mg. of 3α,17α-dihydroxypregnane-11,20-dione and a total of 23,600 c.p.m. Recrystallization from ethyl acetate and acetone produced 7 mg.[4]—45 c.p.m. per mg. Further recrystallization from ethyl acetate resulted in 3 mg. of 3α,17α-dihydroxypregnane-11,20-dione—31 c.p.m. per mg. The crystals remaining after determination of specific activity and the mother liquors of the last 2 recrystallizations were combined and acetylated with pyridine and acetic anhydride. 3α-Acetoxy-17α-hydroxypregnane-11,20-dione (13 mg.) was recrystallized from methanol to yield 10 mg.—108 c.p.m. per mg. The crystals and the mother liquor were recombined (11 mg.) and chromatographed on 1.1 Gm. of silica gel. Elution with 20 per cent ethyl acetate in benzene afforded 5 mg. of product. Re-

[1] The radioactivity was measured in a windowless flow gas counter and the results are corrected to the infinitely thin range. The results are given in counts per minute (c.p.m.). The authors are grateful to their colleague, Dr. H. Leon Bradlow, for these measurements.

[2] Hydrocortisone-4-C^{14} was obtained from the Endocrinology Study Section of the United States Public Health Service.

[3] Ketodase, obtained from the Warner-Chilcott Laboratories, a division of Warner-Lambert Pharmaceutical Company, New York, N. Y.

[4] The melting points of the recrystallized compounds are not reported, although they have been determined and are identical with those of our synthetic samples. Since large amounts of pure compounds were added as carrier and the chromatographic fractions were assayed by paper chromatography and infrared spectrometry, there is no question as to their identity. Most trace contaminants should be eliminated by chromatography and repeated recrystallizations of the compounds and their derivative. Furthermore these compounds have double melting points, frequently with ranges in between. Therefore the melting point would be meaningless for determination of purity. The constant specific activity of the steroid and its derivative is the only criterion of its radiochemical purity.

crystallization from acetone-petroleum ether produced 4 mg.—80 c.p.m. per mg. Subsequent recrystallization from methanol yielded 1.2 mg.—38 c.p.m. per mg., corresponding to 42 c.p.m. per mg. as the free steroid. The average specific activity of *3α,17α-dihydroxypregnane-11,20-dione* was 39 c.p.m. per mg., which corresponds to 1400 c.p.m. in the neutral steroid extract or 0.08 per cent of the total radioactivity.

Elution of the original partition column with 7 per cent ethanol in chloroform yielded fractions containing 41 mg. of 11-ketopregnane-3α,17α,20α-triol—96,000 c.p.m. Recrystallization from ethyl acetate and acetone resulted in 18 mg. of steroid—312 c.p.m. per mg. Recrystallization from acetone produced 8 mg.—84 c.p.m. per mg. Another recrystallization from ethyl acetate yielded 3 mg. of 11-ketopregnane-3α,17α,20α-triol—72 c.p.m. per mg. The mother liquors and the crystals remaining after determination of specific activity were combined (11 mg.) and acetylated with pyridine and acetic anhydride. The product obtained was chromatographed on 50 Gm. of silica gel containing 20 ml. of *tertiary* butyl alcohol and developed with methylene chloride to yield 13 mg. of 3α,20α-diacetoxy-17α-hydroxypregnane-11-one. Recrystallization from methanol produced 9 mg.—24 c.p.m. per mg. The specific activity of *11-ketopregnane-3α,17α,20α-triol* was calculated to be 29 c.p.m. per mg., which corresponds to 1000 c.p.m. in the neutral steroid extract, or 0.06 per cent of the total radioactivity.

Elution of the original partition column with 10 per cent ethanol in chloroform yielded fractions containing 34 mg.—85,000 c.p.m. Recrystallization from ethyl acetate-benzene and acetone-benzene produced 20 mg. of steroid—707 c.p.m. per mg. Recrystallization from ethyl acetate with a trace of benzene yielded 11 mg. of pregnane-3α,11β,17α,20α-tetrol—318 c.p.m. per mg. The crystals remaining after determination of the specific activity and the mother liquors of the last 2 recrystallizations were combined (8 mg.) and acetylated with acetic anhydride and pyridine. The product was chromatographed on 50 Gm. of silica gel containing 20 ml. of *tert.* butyl alcohol. Elution with 2 per cent *tert.* butyl alcohol in methylene chloride yielded 7 mg. of pregnane-3α,11β,17α,20α-tetrol 3,20-diacetate. Recrystallization from methanol resulted in 4.5 mg.—56 c.p.m. per mg. Further recrystallization from ethyl acetate-methanol produced diacetate—49 c.p.m. per mg. The average specific activity of *pregnane-3α,11β,17α,20α-tetrol* was calculated to be 65 c.p.m. per mg., which corresponds to 2350 c.p.m. in the neutral steroid extract or 0.14 per cent of the total radioactivity.

Subject F1 was a 63-year-old woman with chronic lymphatic leukemia. Approximately 0.25 mg. of hydrocortisone-4-C^{14} (1.30×10^6 c.p.m.) in 200 ml. of 5 per cent glucose and 2 ml. of ethanol was administered intravenously over a period of thirty minutes. The metabolic fate of hydrocortisone and its transformation to cortols and cortolones were previously studied in this patient (8, 9).

The urine collected during the fourth through sixth hour after the dose of hormone was treated as previously described. The neutral steroid fraction contained 204,000 c.p.m. To a portion of this extract (62,000 c.p.m.) 21.0 mg. of 11-ketopregnane-3α,17α,20α-triol and 42.8 mg. of pregnane-3α,11β,17α,20α-tetrol were added. The extract was chromatographed on 80 Gm. of silica gel containing 32 ml. of ethanol. Elution with 8 per cent ethanol in methylene chloride yielded 19 mg. of slightly impure product—1800 c.p.m. Recrystallization from ethyl acetate and then from acetone produced 8 mg. of *11-ketopregnane-3α,17α,20α-triol* which was devoid of radioactivity. Elution of the partition column with 10 per cent ethanol in methylene chloride yielded fractions containing 41 mg. of pregnane-3α,11β,17α,20α-tetrol—2000 c.p.m. Recrystallization from ethyl acetate and from ethyl acetate-benzene produced 20 mg. of pure *pregnane-3α,11β,17α,20α-tetrol* which was devoid of radioactivity.

DISCUSSION

In the patient with untreated congenital adrenal hyperplasia, a small dose of hydrocortisone-4-C^{14} was administered intravenously over a period of thirty minutes and a complete urine collection was obtained for two days after. Steroid metabolites were isolated from the urine of the first twenty-four hours after administration of the hormone. This urine specimen contained approximately 90 per cent of the administered radioactivity and therefore the results of the analysis yielded an accurate appraisal of the postulated metabolic pathway. After hydrolysis of the urinary conjugates by β-glucuronidase, carrier amounts of the principal metabolites of 21-deoxyhydrocortisone were added to the extract containing the neutral steroids and these were then purified. The compounds added were 11-ketopregnane-3α,17α,20α-triol, pregnane-3α,11β,17α,20α-tetrol and 3α,17α-dihydroxypregnane-11,20-dione. From consideration of the known pathways of steroid transformation, it can be assumed that these 3 metabolites would be the chief end-products if removal of the C-21 hydroxyl group had been a major metabolic transformation of the hydrocortisone molecule. These would similarly be the major products expected if reductive removal of the C-21 hydroxyl group had occurred after reduction of the α,β unsaturated ketone system in ring A.

The results showed that the 3 compounds together accounted for less than 0.3 per cent of the total radioactivity in the neutral steroid extract. The significance of this slight evidence for the postulated transformation is a matter of opinion. We presume that the tiny amount of radioactivity present was due to trace contaminants difficult to remove. This view is supported by the results of a similar study in the patient who did not have adrenal hyperplasia. As before, a tracer dose of hydrocortisone-4-C^{14} was infused intravenously but in this instance only the urine excreted during the fourth through the sixth hour, inclusive, was studied. This sample of urine contained 27 per cent of the total radioactivity given to the patient and this amount was considered to be sufficiently representative of the total metabolism for the purpose of the study. Isolation of 11-ketopregnane-3α,17α,20α-triol and pregnane-3α,11β,17α,20α-tetrol by the carrier technique was accomplished and these compounds were found to be devoid of radioactivity. The small difference between the results of the 2 studies is ascribed to the more than 13-fold amount of radioactivity in the extract from the adrenal hyperplasia patient compared with the "control" leukemic patient. This, together with other details such as the use of mother liquors for the preparation of a derivative, undoubtedly made purification to radiochemical homogeneity very difficult in the initial experiment. Conversely, the smaller amount of extract and radioactivity in the second experiment made the purification simple.

With the reservation that a questionable conversion may be interpreted from the study with the hyperplasia patient, it has been clearly established that reductive removal of the C-21 hydroxyl group of hydrocortisone is an insignificant feature of the metabolism of that hormone. This conclusion holds for normal man as well as the patient with congenital adrenal hyperplasia. The results of Bradlow and Gallagher (7), who showed that the C-11 oxygen function of 11β-hydroxy-Δ^4-androstene-3,17-dione was retained throughout the metabolic transformations of that hormone, are pertinent to this conclusion.

As a result of this study one may conclude that the large amounts of C-21 deoxysteroid metabolites present in the urine of the patient with congenital adrenal hyperplasia do not originate from hydrocortisone or other C-21 oxygenated steroids. It is probable that the precursors are indeed 21-deoxysteroids and these represent incomplete synthesis of the hormone. There is thus no reason as yet to alter the current concept of the cause and consequences of this disorder.

Acknowledgment

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THE PRESENCE OF AN ABNORMAL THYROID-STIMULATING HORMONE IN THE SERUM OF SOME THYROTOXIC PATIENTS*

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ABSTRACT

Pituitary extracts and sera from a variety of animal and human subjects have been tested for thyroid-stimulating hormone (TSH) activity, by measurement of their effect in raising the plasma I^{131} level in thyroxine-treated guinea pigs. Sera from some cases of thyrotoxicosis elicit abnormal TSH responses. The abnormality consists of a marked prolongation of the time course of the response. When U.S.P. Thyrotropin Reference Substance is added to a serum which elicits the abnormal TSH response, the magnitude and time course of the response induced by the mixture indicate that the two components act independently. It is suggested that sera eliciting the abnormal response contain an abnormal form of TSH. This material, which has so far been found only in sera from thyrotoxic patients, may have some relationship to the hyperthyroidism and possibly also to the exophthalmos of thyrotoxicosis.

WHEN investigated for their content of thyroid-stimulating hormone (TSH), some human sera have been found to induce abnormal responses in the TSH assay (1, 2). The assay method, which has been described previously (3, 4), entails measurement of the effect of TSH in releasing I^{131} from the thyroid glands of thyroxine-injected guinea pigs. The release of I^{131} from the guinea pig's thyroid is measured by following the increase in the animal's plasma I^{131} concentration. In the normal response, the I^{131} level in the guinea pig's plasma is higher three hours after the intravenous injection of the TSH than it is sixteen hours after. In the abnormal response, elicited by sera from some cases of thyrotoxicosis, the reverse occurs—the guinea pig's plasma I^{131} level being higher sixteen hours after the intravenous injection of the serum than it is three hours after.

These observations prompted an investigation of:

I. The mechanism of the abnormal response.

II. The type of response elicited by the TSH present in human, bovine

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and rat pituitary glands, and in the blood of thyroidectomized rats and a variety of human subjects.

METHODS

The TSH assay was performed as described previously regarding the preparation and bleeding of the guinea pigs, the injection of the test material, and the counting of the radioactivity in the plasma (4).

Preliminary study of the time course of the normal and abnormal responses indicated that measurements of the guinea pig's plasma I^{131} levels at three hours and sixteen hours after the intravenous injection of the test material would suffice to determine the type of response. Accordingly, the guinea pigs were bled at three hours and at sixteen hours.

The "3-hour response" represents the difference between the animal's plasma I^{131} level immediately before injection of TSH and the level three hours afterwards; in similar fashion the "16-hour response" represents the difference sixteen hours afterwards. Both the 3-hour and the 16-hour responses are expressed as percentages of the guinea pig's plasma I^{131} level immediately before the first injection of TSH. This partially corrects for variation in thyroidal I^{131} uptake between the guinea pigs.

The plasma I^{131} levels of the guinea pigs before the first injection of TSH average about 140 net counts/minute/0.2 ml. of plasma, and rarely exceed the range of 70–200 net counts/minute/0.2 ml.

For the study of the action of a mixture of standard TSH and serum eliciting the abnormal response (Fig. 2), 6 guinea pigs were used. Each animal received, in randomized order, the standard, the serum and the mixture. The 3 injections were made at 48-hour intervals, and the 3-hour and 16-hour responses to each injection were determined (Table 1). It was necessary to make a baseline correction for the second and third injections, as each guinea pig's plasma I^{131} level was still decreasing following the response to the previous injection. This was accomplished by extrapolating the rate of fall of the guinea pig's plasma I^{131} level in the thirty-two hours between the pre-injection bleeding and the 16-hour bleeding for the previous injection. It was assumed that the rate of fall was exponential.

The primary object of the study of the pituitary extracts and the rat plasma (Table 2) was to determine the type of response they would elicit. Groups of 3–6 animals were given injections of the substance being tested, and the 3-hour and 16-hour responses were measured; the animals were used two or three times at 48-hour intervals. No comparison with a standard TSH preparation was made, so the sensitivity of the animals remains unknown, but previous experience has indicated that this does not vary widely. Therefore, as the 3-hour responses are comparable with those used for plotting the dose-response relationship of the assay (4), rough estimates of the amounts of normal TSH present were obtained by making reference to the dose-response curve.

In the study of human sera (Table 3) it was necessary to use control injections of saline, as many of these sera contain amounts of TSH which are near the limit of detection by the assay. Moreover, the baseline plasma I^{131} levels of the guinea pigs undergo slight fluctuations which, although not very important over the short interval of the 3-hour response, are more important over the longer interval of the 16-hour response. Guinea pigs given injections of saline were bled three hours and sixteen hours later, as were the animals given injections of the serum being tested, and the 3-hour and 16-hour "responses" to saline (usually negative) were determined. The values listed in Table 3 are the differences between the responses elicited by the serum and those observed with the saline controls. When the difference is statistically significant ($P < 0.05$), the re-

sponse to the serum is listed in heavy type. Equal numbers of injections of serum and saline were made, in randomized order, each guinea pig receiving a total of 3 injections at 48-hour intervals.

To determine the type of response obtained, the 3-hour value was divided by the 16-hour value to yield the "response ratio." The ratio, 2.38, given by 1.0 mU of U. S. Pharmacopeia Thyrotropin Reference Substance (see Table 1) was taken as the standard for a "normal" response. The ratio, 0.36, given by 0.5 ml. of serum from thyrotoxic patient M.McC. (see Table 1), was taken as the standard for an "abnormal" response.

The types of response were defined as follows:

A "significant" TSH response is one in which the 3-hour or the 16-hour value (or both) is significantly greater ($P < 0.05$) than the saline control value.

A "normal" TSH response is a significant response with a ratio, the fiducial limits of which ($P = 0.05$) include the normal ratio, 2.38, but exclude the abnormal ratio, 0.36.

An "abnormal" TSH response is a significant response with a ratio, the fiducial limits of which ($P = 0.05$) include the abnormal ratio, 0.36, but exclude the normal ratio, 2.38.

TABLE 1. THE RESPONSES ELICITED BY SERUM FROM A PATIENT WITH THYROTOXICOSIS AND EXOPHTHALMOS (M.McC.), BY 1.0 mU OF U.S.P. THYROTROPIN REFERENCE SUBSTANCE, AND BY THE TWO MIXED TOGETHER

The responses are baseline-corrected and are expressed as percentages of the plasma I^{131} level obtaining immediately before the first injection of TSH in the assay. The response ratio is the 3-hour response divided by the 16-hour response. The fiducial limits of the response ratio are for $P = 0.05$.

Material assayed	Percentage Increase in Guinea-Pig Plasma I^{131} Level					
	0.5 ml. M.McC. serum		1.0 mU U.S.P. TSH		0.5 ml. M.McC. serum + 1.0 mU U.S.P. TSH	
Time of response	3-hr. r1	16-hr. r2	3-hr. r1	16-hr. r2	3-hr. r1	16-hr. r2
Guinea pig 1	91	231	278	122	414	585
2	60	126	173	79	198	318
3	90	263	152	73	170	135
4	70	213	254	38	169	116
5	81	240	124	46	—*	—*
6	39	127	149	115	244	328
Σ	431	1200	1130	473	1195	1482
$\bar{x}$	72	200	188	79	239	296
Response ratio r1 ÷ r2	0.36		2.38		0.81	
l_1 r1 ÷ r2	0.46		3.31		1.07	
l_2 r1 ÷ r2	0.27		1.82		0.60	

* Values missing due to accidental death of guinea pig.

An "uncertain" TSH response is a significant response with a ratio, the fiducial limits of which (P = 0.05) include both the normal, 2.38, and the abnormal, 0.36, ratios.

A "mixed" TSH response is a significant response with a ratio, the fiducial limits of which exclude both the normal, 2.38, and the abnormal, 0.36, ratios.

The method of calculating the fiducial limits of the response ratios is described in the Appendix.

All injections of materials being assayed were made intravenously, in a volume of 1.0 ml.

When it was necessary to store sera, they were either freeze-dried or stored in the frozen state.

RESULTS

I. *The Mechanism of the Abnormal Response*

(a) *The time course of normal and abnormal responses*

Figure 1 shows the time course of the responses elicited by a standard TSH preparation (U. S. Pharmacopeia Thyrotropin Reference Substance) and by serum from a case of thyrotoxicosis with exophthalmos. Three animals were used for each of the 2 doses of standard, the values shown

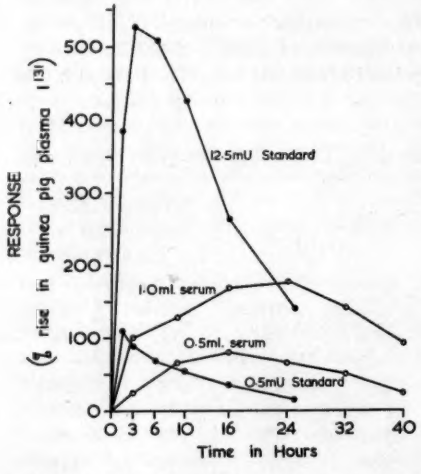

FIG. 1. Time courses of the responses to 2 doses of standard TSH and to 2 doses of serum from a case of thyrotoxicosis with exophthalmos.

The standard TSH was U. S. Pharmacopeia Thyrotropin Reference Substance, in doses of 12.5 mU and 0.5 mU. The doses of serum were 1.0 ml. and 0.5 ml. The curves for the 2 doses of standard each represent the mean values in 3 animals. The curves for the 2 doses of serum each represent values in a single animal.

being the means. The values for the 2 doses of serum were each obtained in 1 animal only, insufficient serum being available to permit the use of groups of animals.

With the dose of 12.5 mU of standard, the highest point recorded in the response was at three hours after the intravenous injection, when the value was about twice that at sixteen hours. With the dose of 0.5 mU of standard, the highest point recorded was at 1½ hours, when the value was about three times that at sixteen hours.

The serum used was that from a woman with exophthalmos and an

untreated recurrence of thyrotoxicosis; she had had a subtotal thyroidectomy several years before. Results of an I^{131} tracer test were typical of thyrotoxicosis (5), namely, 74 per cent of the dose in the thyroid at eight hours and 61 per cent at forty-eight hours.

With the dose of 1.0 ml. of serum, the highest point recorded in the response was at twenty-four hours, when the value was 1.8 times as great as at three hours. With the dose of 0.5 ml. of serum, the highest point recorded was at sixteen hours, when the value was about three times as great as at three hours.

With both the standard TSH and the serum, the smaller doses produced their maximum response earlier than did the larger doses. This effect, however, was small compared with the gross difference in time courses between the standard TSH and the serum. It is apparent that there are no doses of standard thyrotropin which could elicit responses of the same magnitude and time course as those elicited by the serum.

(b) *Relationship to the frequent toxicity of human serum when injected into guinea pigs*

Human serum is often toxic to guinea pigs. The degree of toxicity varies from serum to serum, the manifestations ranging from a transient deepening and slowing of respiration, to death of the animal within a few minutes.

The first human serum to elicit the abnormal TSH response was moderately toxic, and it was thought that this toxic effect might be the cause of the unusual time course of the response. From Table 3, however, it is seen that there was no correlation between the type of TSH response and the presence or absence of toxicity. Moreover, the toxicity could be abolished by dialysis against a dextran-saline solution, which affects neither the magnitude nor the time course of the TSH response.

(c) *The action of a mixture of standard TSH and serum eliciting the abnormal response*

The finding that the abnormal response was not related to the frequent toxicity of human serum for guinea pigs does not exclude the possibility that the abnormality might be due to the presence in some sera of a substance which modifies the time course of action of normal TSH. That this was not so can be seen from Table 1 and Figure 2, which show that when standard TSH was added to a serum which induced the abnormal response, the mixture elicited 3-hour and 16-hour responses which closely approximated the arithmetical sums of the responses to the standard and serum given separately. It is apparent that the two types of response can occur independently and simultaneously.

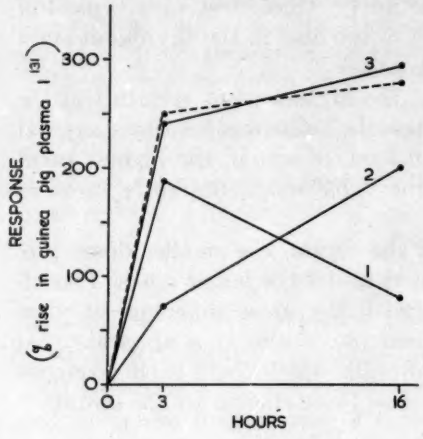

FIG. 2. The 3-hour and 16-hour responses to standard TSH, to serum from a case of thyrotoxicosis with exophthalmos (M.McC.), and to a mixture of the 2 materials.

The curves are drawn from the data in Table 1:

Curve 1—1.0 mU of U.S. Pharmacopeia Thyrotropin Reference Substance (U.S.P. TSH). Mean of 6 responses.

Curve 2—0.5 ml. of serum from a case of thyrotoxicosis with exophthalmos (M.McC.). Mean of 6 responses.

Curve 3—1.0 mU of U.S.P. TSH+0.5 ml. of M.McC. serum. Mean of 5 responses.

Broken Curve—arithmetical sum of Curve 1 and Curve 2.

The response ratio (the 3-hour response divided by the 16-hour response) for the serum was 0.36, for the standard it was 2.38, and for the mixture it was 0.81. The fiducial limits ($P=0.05$) of the response ratio of the mixture were clear of the limits for both the serum and the standard (Table 1).

(d) *Effect of massive dosage of thyroxine on the abnormal TSH response*

The guinea pigs used in the TSH assay were given injections of 40 μg. of *l*-thyroxine daily in order to inhibit the secretion of TSH from their own pituitary glands. It would be possible for the abnormal TSH response to be due to blockage of the inhibitory action of thyroxine, thus causing stimulation of the guinea pig's thyroid by TSH secreted from the animal's own pituitary. To test this possibility, 1 mg. of *l*-thyroxine was injected subcutaneously into 2 test guinea pigs, thirty minutes before intravenous injection of 1.0 ml. of a serum known to elicit the abnormal response. The mean 3-hour response in the 2 animals was 36 per cent and the mean 16-hour response was 156 per cent. These responses did not differ significantly from those in 2 other guinea pigs not receiving the large dose of thyroxine, in which the mean 3-hour and 16-hour responses were 32 per cent and 155 per cent respectively. This makes it improbable that the abnormal response was due to blockage of the thyroxine-inhibition of endogenous TSH secretion from the test guinea pig's own pituitary gland.

II. *Type of Response Elicited by TSH from Various Sources*

(a) *Pituitary glands*

The preparations assayed were:

1. A saline extract of the anterior lobe of a human pituitary, taken from a patient with status asthmaticus.
2. A saline extract of normal rat pituitary.
3. The U. S. Pharmacopeia Thyrotropin Reference Substance, derived from the pituitaries of cattle.
4. An acetone-dried extract of mouse pituitary tumor (Furth, strain 101D, generation 0).

From Table 2 it is seen that all these pituitary extracts induced the normal TSH response.

(b) *Rat plasma*

From Table 2 it is seen that the plasma of thyroidectomized rats elicited the normal TSH response. A significantly smaller response ($P<0.01$) was

TABLE 2. THE TYPE OF TSH RESPONSE ELICITED BY HUMAN, RAT AND BOVINE PITUITARY GLANDS, BY A MOUSE PITUITARY TUMOR AND BY THE PLASMA OF THYROIDECTOMIZED RATS

The response ratio is the 3-hour response divided by the 16-hour response. The amounts of TSH stated to be present are rough estimates only, obtained by making reference to the dose-response curve.

Materials Tested	Dose per injection	No of responses measured	Mean response		Response ratio	Lower limit of response ratio, for P=0.05	Estimated milli-units of TSH per injection	Estimated milli-units of TSH per mg. or ml.	Type of response
			3-hr.	16-hr.					
(a) *Pituitary Glands*									
1. Human pituitary (case of status asthmaticus)	312 μg.	5	261	100	2.61	1.21	3.5	11	N*
2. Rat pituitary	45 μg.	5	144	53	2.72	1.01	1.3	29	N
3. Bovine pituitary	2 μg.	19	9.6	2.4	4.0	0.71	0.11	55	N
(U.S. Pharmacopeia Thyrotropin Reference Substance; 20 mg = 1.0 I.U.)	8 μg.	12	44	12	3.67	1.14	0.37	46	N
	20 μg.	6	159	59	2.69	1.59	1.60	80	N
4. Mouse pituitary tumor. (Furth, strain 101D, generation O)	200 μg.	6	280	110	2.55	1.02	3.9	19.5	N
(b) *Rat Plasma*									
1. Plasma from thyroidectomized rats	1.0 ml.	5	342	126	2.71	1.57	5.5	5.5	N
2. Plasma from thyroxine-injected thyroidectomized rats	1.0 ml.	4	25				0.2	0.2	

* N = normal TSH response.

induced by plasma taken from thyroidectomized rats twenty-four hours after the injection of *l*-thyroxine into the rats in a dosage of 10 μg. per 100 Gm. of body weight.

Unlike human serum, rat plasma and serum are never toxic to the guinea pigs.

(c) *Human serum*

Table 3 shows the mean 3-hour and 16-hour responses elicited by sera from human subjects in various thyroidal states.

1. *Normal euthyroid subjects.* It is seen that no TSH was detected in the serum of 4 normal euthyroid subjects.

2. *Congenital hypothyroidism.* Normal TSH responses were elicited by sera from 4 patients with congenital hypothyroidism, 3 being cretinous babies (B.F., L.G. and B.McC.) and 1 being a girl of 11 years (G.B.).

The mean response ratio of these 4 hypothyroid cases was 2.30, which closely approximated the mean response ratio of 2.38 associated with U. S Pharmacopeia Thyrotropin Reference Substance (Table 1).

No TSH was demonstrable in the serum of either B.F. or G.B. after they had been receiving thyroxine therapy for several months. Later, G.B.'s thyroxine therapy was stopped for two weeks and at the end of this time her serum elicited a small significant response. The response ratio was 2.60, but the fiducial limits included the abnormal ratio, so the type of response was uncertain.

3. *Thyrotoxicosis.* Of sera from 5 cases of untreated thyrotoxicosis with exophthalmos, 2 (J.P. and B.A.) elicited no response; 1 (V.C.) induced a "super-abnormal" response; 1 (A.C.), an abnormal response; and 1 (A.S.), an uncertain type of response. The upper limit ($P=0.05$) of V.C.'s response ratio was 0.31, which was lower than the standard abnormal response ratio of 0.36. This was the only super-abnormal response observed, and it was included in the abnormal group. There were no "super-normal" responses.

In the untreated thyrotoxic group, the mean response ratio for the 3 sera which induced significant responses was 0.38, which closely approximated the standard abnormal response ratio of 0.36.

The administration of 0.6 mg. of *l*-thyroxine daily to Patient A.C. for two weeks did not reduce the size of the response to her serum; rather it increased somewhat, and the type of response remained abnormal.

A very large abnormal response was induced by the serum of Patient M.McC., who had severe exophthalmos and a recurrence of thyrotoxicosis. Several years before, a previous episode had been effectively treated by subtotal thyroidectomy. This is the serum which was used for the experiment shown in Table 1. Table 3 shows the mean responses to a dosage of

TABLE 3. THE TYPE OF TSH RESPONSE ELICITED BY SERA FROM PATIENTS WITH VARIOUS THYROID CONDITIONS

The response ratio is the 3-hour response divided by the 16-hour response. The amounts of normal TSH stated to be present are rough estimates only, obtained by making reference to the dose-response curve. When values for responses are in heavy type, they differ significantly from the saline control values; when they are in ordinary type, they do not.

Clinical status	Patient	No. of responses measured	Toxic to guinea pigs	Mean response		Response ratio	Upper limit of response ratio, for P=0.05	Lower limit of response ratio, for P=0.05	Estimated milli-units of TSH per 1 ml. serum	Type of response
				3-hr.	16-hr.					
1. *Normal euthyroid subjects*	D.A.	7	yes	6	0					none
	W.C.	5	no	1	− 1					none
	L.B.	5	no	6	−17					none
	M.S.	5	no	−1	−11					none
Mean				3.3	−6.6					
2. *Congenital hypothyroidism*										
(a) Untreated	B.F.	5	yes	**216**	**103**	2.10	3.65	1.39	2.5	N*
	L.G.	4	yes	**181**	**81**	2.23	5.49	1.27	2.0	N
	B.McC.	2	yes	**217**	**101**	2.15	7.77	1.10	2.5	N
	G.B.	3	no	**121**	30	4.03		1.03	1.0	N
Mean				**186**	**81**	2.30				
(b) Receiving thyroxine	B.F.	2	yes	−11	−11					none
	G.B.	3	no	4	3					none
Mean				−2	−3					
(c) No thyroxine for 2 wks.	G.B.	17	yes	**13**	**5**	2.60			0.15	?*
3. *Thyrotoxicosis*										
(a) Thyrotoxicosis with exophthalmos										
i) Untreated	J.P.	5	yes	3.5	−10					none
	B.A.	4	no	−2	1					none
Mean				1.1	−5.1					
	V.C.	8	no	−2	**19**	−0.11	0.31			A*
	A.C.	6	no	**23**	**52**	0.44	1.53			A
	A.S.	8	yes	**14**	**20**	0.70				?
Mean				**10.6**	**28**	0.38				
ii) Receiving thyroxine	A.C.	4	no	**26**	**78**	0.33	0.67	0.06		A
iii) Recurrence of thyrotoxicosis after thyroidectomy	M.Mc.C	6	yes	**192**	**560**	0.34	0.64	0.09		A
iv) Euthyroid after thyroidectomy	V.C.	7	no	−6	10					none
v) Euthyroid after I^{131} therapy	J.P.	6	yes	**25**	**60**	0.42	1.10			A
vi) Grossly hypothyroid due to methylthiouracil	J.G.	10	yes	**99**	**78**	1.27	7.00	0.40	0.75	N
(b) Thyrotoxicosis without exophthalmos										
i) Untreated	P.McC.	11	yes	1	1					none
ii) Euthyroid, after thyroidectomy	P.McC.	11	yes	**14**	**21**	0.67	1.56	0.18		A
iii) Grossly hypothyroid due to methylthiouracil	O.B.	5	no	**47**	**20**	2.35		0.94	0.4	N

* N=normal TSH response; A =abnormal TSH response; ? =uncertain type of TSH response.

1.0 ml. of M.McC.'s serum. The response ratio, 0.34, closely approximated the ratio 0.36 shown in Table 1, when a dosage of 0.5 ml. of M.McC.'s serum was used.

A second serum sample from Patient V.C., taken when she was euthyroid several months after a subtotal thyroidectomy, elicited no significant response.

A second serum sample from Patient J.P., taken when he was euthyroid several months after I^{131} therapy, induced an abnormal response, in contrast to lack of response to the serum sample taken before treatment.

A normal TSH response was elicited by the serum of Patient J.G., who had thyrotoxicosis initially but who had been rendered grossly hypothyroid by overdosage with methylthiouracil.

Five of these patients, J.P., B.A., A.C., M.McC. and J.G., had severe exophthalmos. The other 2 (V.C. and A.S.) had mild exophthalmos. The degree of exophthalmos in J.P. and V.C. had not changed in the time intervals between the collection of their first and second serum samples.

No response was elicited by the serum of Patient P.McC., who had thyrotoxicosis (untreated) without exophthalmos. She had a moderate to large diffuse goiter of recent origin. Later when she was euthyroid following a subtotal thyroidectomy, her serum induced a response of the abnormal type.

Serum from another thyrotoxic patient without exophthalmos (O.B.), who had been rendered grossly hypothyroid by overdosage with methylthiouracil, elicited a normal response. This patient had a large multinodular goiter of long standing.

III. *Frequency Distribution of the Materials Tested When Classified According to the Type of TSH Response Elicited*

Figure 3 is constructed from the data in Tables 2 and 3. It is seen that pituitaries and blood samples from sources other than cases of thyrotoxicosis, induced only normal TSH responses, except for 1 instance in which a weak human serum (G.B.) elicited an uncertain type of response.

In contrast to this is the frequency distribution of sera from cases of thyrotoxicosis. There were 6 abnormal responses, 1 uncertain response and 2 normal responses. The normal responses were both induced by sera from thyrotoxic patients who had been rendered grossly hypothyroid by an antithyroid drug.

DISCUSSION

The size of the abnormal response was not reduced by injection into the test guinea pig of a massive dose of thyroxine. Therefore, it is unlikely that the abnormal TSH response is due to blockage of the thyroxine-

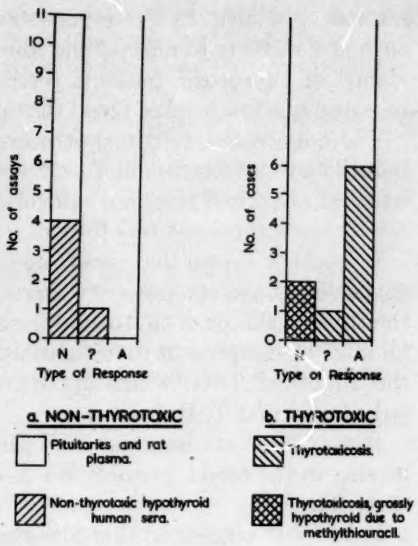

FIG. 3. The frequency distribution of materials tested, according to the type of TSH response. (**a**) Materials from sources other than patients with thyrotoxicosis, namely, human pituitary, rat pituitary, bovine pituitary (U.S.P. TSH at 3 dosages), mouse pituitary tumor, plasma from thyroidectomized rats and sera from cases of congenital hypothyroidism. (**b**) Sera from cases of thyrotoxicosis.

N = normal TSH response; A = abnormal TSH response; ? = uncertain type of TSH response.

inhibition of endogenous TSH secretion from the test guinea pig's own pituitary gland. This does not exclude the possibility that the abnormal response is due to stimulation of the guinea pig's pituitary. However, it seems most likely that the action is directly on the thyroid.

The finding that the action of standard TSH was not affected by its addition to a serum eliciting the abnormal response, means that the abnormal response cannot be attributed to interference with the action of normal TSH. It is probable, therefore, that the abnormal response is a manifestation of the presence of an abnormal thyroid-stimulating hormone.

The finding of abnormal responses only to sera from cases of thyrotoxicosis makes it seem likely that the abnormal TSH has some relationship to this disorder. However, the data collected so far are not adequate to exclude with certainty the possibility that abnormal TSH may occur in conditions other than thyrotoxicosis.

If the 2 thyrotoxic patients who were hypothyroid due to methylthiouracil are excluded, then there is a clear gap between the response ratios of the thyrotoxic and of the non-thyrotoxic groups. The highest response ratio in the thyrotoxic group was 0.70 (A.S.) and the lowest in the non-thyrotoxic group was 2.10 (B.F.).

Of the 2 thyrotoxic patients (O.B. and J.G.) who were hypothyroid due to methylthiouracil, O.B's response ratio of 2.35 approximated the normal standard value, but J.G.'s ratio of 1.27 lay between that for the thyrotoxic and non-thyrotoxic groups. Its lower fiducial limit just clears the ab-

normal standard. J.G.'s response ratio could be indicative of the presence of a mixture of normal and abnormal TSH. It may be that pituitary glands of thyrotoxic patients secrete normal TSH in the usual way in response to a low level of blood thyroxine.

The only mixed TSH response (as defined under "Methods") was that induced by the mixture of U.S.P. standard and M.McC. serum. The normal and abnormal response categories, however, could include mixtures in which one component was present in predominant amount.

It has been shown that sera from some cases of exophthalmos and hyperthyroidism have the power to increase markedly the acinar cell height of the thyroid glands of thyroxine-injected guinea pigs (6). In the light of the findings of the present investigation, it is likely that these sera contained the abnormal TSH, which apparently affects thyroid morphology similarly to normal TSH.

It may be that abnormal TSH differs from the normal in the length of its stay in the blood; possibly it is normal TSH combined with some other material.

It has been suggested that although TSH has been demonstrated in the serum of only some cases of thyrotoxicosis, in all cases of this disease there may be a higher level than in normal euthyroid subjects, in whom the level is too low to be measured reliably (7). Similarly, although the abnormal TSH has been demonstrated only in some cases of thyrotoxicosis, it may be present in all cases at a level too low to be detected by the present assay procedure. It is possible, therefore, that the abnormal TSH plays a causative role in the hyperthyroidism of thyrotoxicosis. It may also be implicated in the etiology of exophthalmos. It is possible that the "exophthalmos-producing substance" found by Dobyns and Wilson (8) in the serum of cases of progressive exophthalmos, is the abnormal TSH.

The site of production of the abnormal TSH remains unknown, but suspicion naturally falls on the pituitary. It would be of great interest to determine the type of TSH present in the pituitary gland of a patient with thyrotoxicosis.

Acknowledgments

I am indebted to Dr. H. D. Purves, Principal Research Officer of this laboratory, for most helpful criticism and for the method of determining the fiducial limits of the response ratios.

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APPENDIX

(by H. D. Purves)

The problem of finding the fiducial limits of the ratio between 2 independent variates does not appear to have received much attention. None of the standard textbooks on statistical methods for research workers refers to it. The problem resolves itself into transforming the ratio into a function which has an approximately normal distribution. In this instance this has been done by use of the "ratio angle" defined as the angle with a tangent equal to the ratio of the 2 variates. Thus if x and y are the 2 variates, the ratio angle is defined by $\theta = \tan^{-1} x/y$.

In estimating variance, the procedure of "Student's" *t*-test is followed in assuming equal variance in the measurements of x and of y. A combined estimate of variance is obtained by the formula

$$s^2 = \frac{\Sigma(x - \bar{x})^2 + \Sigma(y - \bar{y})^2}{2n - 2}$$

where

$\bar{x}$ = mean of n values of x;
$\bar{y}$ = mean of n values of y; and
$2n-2$ = degrees of freedom used in estimating variance.

In these circumstances the standard deviations of $\bar{x}$ and of $\bar{y}$ are equal and are estimated by the expression $\frac{s}{\sqrt{n}}$.

It can be shown that in this case the estimates of θ, denoted by $\theta_s = \frac{\bar{x}}{\bar{y}}$, are symmetrically distributed about the true value of θ with a distribution given by the expression:

$$\theta_s - \theta = 0 \pm \mathrm{Sin}^{-1} \frac{\sigma}{\sqrt{n(x^2 + y^2)}}$$

in which x and y are the population values of mean responses and σ^2 the population variance. Substituting the sample values of these quantities, we have an estimate of the error in θ_s as given by:

$$\delta\theta = \theta_s - \theta = \pm \operatorname{Sin}^{-1} \frac{s}{\sqrt{n(\bar{x}^2 + \bar{y}^2)}}$$

An estimate of the limits of $\delta\theta$ for $P = 0.05$ is obtained by

$$\delta\theta = \pm \operatorname{Sin}^{-1} \frac{ts}{\sqrt{n(\bar{x}^2 + \bar{y}^2)}}$$

where t is the value given for $P = 0.05$ for $2n-2$ degrees of freedom in the table of t (9). This estimate is only valid when $(\bar{x}^2+\bar{y}^2)$ is equal to the population value of (x^2+y^2). Errors in $\bar{x}$ and $\bar{y}$ cause $\delta\theta$ to be sometimes underestimated and sometimes overestimated. There is no easy way of correcting for this effect; nevertheless the estimate so obtained may be used as an objective estimate of the fiducial limits of the ratios observed and one which approximates to the true limits when $\bar{x}$ and $\bar{y}$ are highly significant, *i.e.*, when they are much greater than their standard errors. This means that the fiducial limits of θ are accurately estimated only when these limits are close, *i.e.*, when

$$\frac{ts}{\sqrt{n(\bar{x}^2 + \bar{y}^2)}} \ll 1.$$

When

$$\frac{ts}{\sqrt{n(\bar{x}^2 + \bar{y}^2)}}$$

is not small, not only are the limits of θ wide but they are also inaccurately estimated, whereas when

$$\frac{ts}{\sqrt{(\bar{x}^2 + \bar{y}^2)}} \lesssim 1,$$

there is no limit to the value of θ and the ratio is not significant.

In tests of responses with saline controls, the 3-hour and 16-hour responses are derived from the differences between responses to saline and responses to test material. In this case

$$s^2 = \frac{\Sigma(a - \bar{a})^2 + \Sigma(b - \bar{b})^2 + \Sigma(c - \bar{c})^2 + \Sigma(d - \bar{d})^2}{4n - 4}$$

$a_1\ a_2 \cdots, a_n$ = 3-hr. responses to test.
$b_1\ b_2 \cdots, b_n$ = 3-hr. responses to saline.
$c_1\ c_2 \cdots, c_n$ = 16-hr. responses to test.
$d_1\ d_2 \cdots, d_n$ = 16-hr. responses to saline.
$4n - 4$ = degrees of freedom used in estimating variance.

$$x = \text{3-hr. response} = \bar{a} - \bar{b}$$
$$y = \text{16-hr. response} = \bar{c} - \bar{d}$$
$$\theta = \tan^{-1} \frac{x}{y}$$
$$\delta\theta = \operatorname{Sin}^{-1} \frac{ts\sqrt{2}}{\sqrt{n(x^2 + y^2)}}$$

= value with $P = 0.05$ for $4n - 4$ degrees of freedom (9).

Then fiducial limits of $\frac{x}{y} = \tan(\theta+\delta\theta)$ and $\tan(\theta-\delta\theta)$.

THE METABOLISM OF 3:3′:5′-TRIIODOTHYRONINE IN MAN*

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ABSTRACT

I^{131}-labeled 3:3′:5′-triiodothyronine was administered intravenously to 10 euthyroid hospital patients. Disappearance from the serum and accumulation in the urine were measured. 3:3′:5′-Triiodothyronine was rapidly degraded and its iodide appeared as free iodide in the serum. The mean serum half-time of the injected compound was of the order of thirty minutes. Within twenty-four hours, more than 50 per cent of the administered labeled iodine was usually eliminated in the urine. Practically all was free iodide. Only a slight amount was excreted as the parent compound. No intermediate degradation products were found in the blood or urine. The rapid metabolic degradation of 3:3′:5′-triiodothyronine makes improbable its existence as a naturally occurring thyroid hormone in man.

IN RECENT years there has been much interest in the natural occurrence and biologic activity of the iodinated thyronines. 3:5:3′:5′-Tetraiodothyronine (thyroxine) and 3:5:3′-triiodothyronine are normal constituents of thyroglobulin and plasma and are metabolically active thyroid hormones. In addition to these, Roche and his associates (1) have reported that 3:3′-diiodothyronine is a naturally occurring thyroid hormone in the rat. This substance is said to account for 25 per cent of the iodine formerly attributed to thyroxine in thyroglobulin (1) and in serum (2). It has recently been shown in man, however, that the iodine of injected 3:3′-diiodothyronine is rapidly excreted in the urine as iodide (3). Thus, it is improbable that it exists in significant concentration in the blood under normal conditions. 3:3′:5′-Triiodothyronine (3:3′:5′-Th) has also been identified by Roche *et al.* in rat thyroglobulin (1) and serum (4). In the rat, urinary and fecal excretion of administered 3:3′:5′-Th is said to be similar to that of injected thyroxine (5), although the antigoitrogenic effect of this compound is only 5 per cent as great as that of thyroxine (6).

The present study records the fate of 3:3′:5′-Th after intravenous administration to 10 euthyroid patients.

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MATERIALS AND METHODS

I^{131}-labeled 3:3′:5′-Th was prepared by iodination of *dl*-3-monoiodothyronine. Then 2.7 mg. of NaI and 5 mc. of I^{131} were acidified with 0.1 ml. of 1 *N* HCl and oxidized with 0.4 ml. of 0.6 per cent H_2O_2. Iodine was slowly collected in 2 ml. of ether. The water layer was discarded and 500 μg. of *dl*-3-monoiodothyronine in 1 ml. of ethanol-ammonia were added. The solution was made alkaline with 0.1 *N* KOH, and after six hours at room temperature was dried by air stream. The residue was dissolved in ethanol-ammonia and applied to Whatman No. 1 paper for descending chromatography in a butanol-3 *N*-ammonium hydroxide solvent system. Flanking strips carried 3:3′:5′-Th and iodide. After chromatographic resolution the flanking strips were dried and stained for iodide with palladium chloride and for 3:3′:5′-Th with diazotized sulfanilic acid or with Ninhydrin. On the chromatogram of the synthesis products, the area corresponding to the flanking 3:3′:5′-Th was cut out and eluted in 0.9 per cent NaCl made alkaline with 0.1 *N* KOH. In eluting, care was taken to exclude 3:3′-Th, which was produced in small but significant quantities in this synthesis. The yield of 3:3′:5′-Th was from 1 to 5 per cent. The final preparation was tested by chromatography in all or most of the following solvent systems: butanol-3 *N* ammonium hydroxide, isopentanol-2 *N* ammonium hydroxide, butanol-2 *N* acetic acid, and methanol-0.2 *M* ammonium acetate. Carrier solutions of 3:3′:5′-Th, 3:5:3′-triiodothyronine, thyroxine, 3:3′-diiodothyronine, 3:5-diiodothyronine, 3-monoiodothyronine, diiodotyrosine, monoiodotyrosine, and sodium iodide were used. The preparation for injection was used for injection only when significant labeling occurred exclusively in the 3:3′:5′-Th and iodide spots, as shown by control chromatographic analysis. The final preparation contained free iodide in amounts ranging from 0 to 12.2 per cent. Representative samples of labeled 3:3′:5′-Th were also chromatographed in the several solvent systems noted, and radioautograms prepared. These indicated that I^{131} was present only as iodide and 3:3′:5′-Th. The R_f values corresponded closely to those of Roche (1).

Patients were selected from the medical and surgical wards. All were clinically euthyroid at the time of study and were without history of thyroid abnormality. Most of the studies were carried out on the metabolic ward to insure accurate urine collections. Each patient received intravenously a known quantity of I^{131}-labeled 3:3′:5′-Th in amounts ranging from 16 to 60 μc. in 7 to 15 ml. of normal saline. An aliquot was saved for a counting standard, for chromatography, and for I^{127} determination. The quantity of stable 3:3′:5′-Th in the injections was less than 5 μg.

Samples of blood and urine were obtained as indicated in the accompanying tables and charts. Measurement of labeled iodine in serum and urine was carried out using 2-ml. samples in a well-type scintillation crystal detector with pulse height analyzer. For determination of labeled iodide and 3:3′:5′-Th content, 2-ml. samples of serum were acidified to pH 2 with 5 per cent H_2SO_4 and extracted three times with butanol in equilibrium with 5 per cent $Na_2S_2O_3$. The extracts were combined and shown to contain well over 90 per cent of the initial labeled iodine. They were then dried by air stream and the residue dissolved in ethanol-ammonia and applied to Whatman No. 3 chromatography paper. Most urine specimens contained sufficient labeled iodine to permit direct application to the paper; the rest were extracted with butanol. All chromatograms were marked with 3:3′:5′-Th, NaI, 3:5:3′-triiodothyronine, 3:3′-diiodothyronine, 3:5-diiodothyronine, and 3-monoiodothyronine. Chromatography routinely was carried out in a butanol-3 *N* ammonium hydroxide solvent system. The chromatograms were stained, cut according to zone of interest, and measured for labeled iodine. A small correction was made for labeled iodine found in areas of the paper not corresponding to

the stained markers. This "paper background" was usually less than 0.4 per cent of the total counts on paper per centimeter.

RESULTS

The data showing the disappearance of labeled iodine from the serum and its appearance in the urine are listed in Tables 1 and 2. The data for 3 patients whose studies were complete for twenty-four hours are charted in Figures 1 and 2. After one hour the amount of labeled iodine in the serum was approximately one-quarter its value at five minutes. Urinary excretion of the injected labeled iodine varied considerably with the individual subject; most subjects excreted 10 to 30 per cent in the first four hours and over 50 per cent by the end of twenty-four hours.

3:3′:5′-Th was rapidly degraded to free iodide in the serum. This was measured in 7 of the subjects. The data appear in Table 2 and are charted in Figure 3. A small correction has been applied to the data of Figure 3 for the free iodide which was present in the injection solution. The ratio

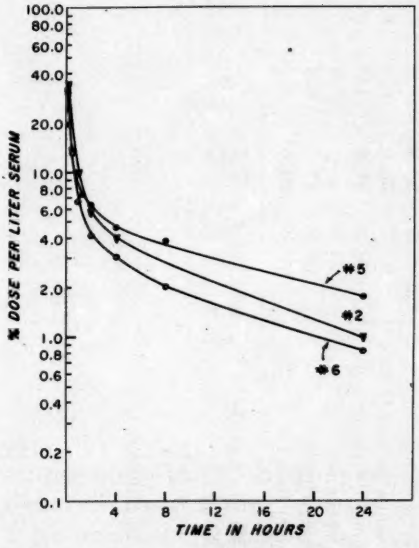

FIG. 1. Disappearance of total I^{131} from serum after intravenous injection of I^{131}-labeled 3:3′:5′-Th in 3 patients (Nos. 2, 5, and 6).

The abscissa shows time in hours. The ordinate denotes the percentage of the dose per liter of serum, plotted logarithmically.

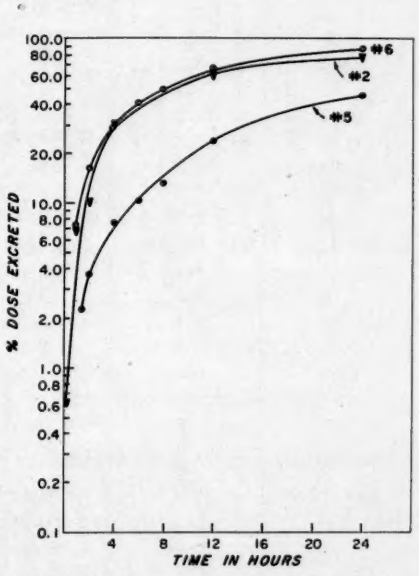

FIG. 2. Accumulation of total I^{131} in urine in the 3 patients of Figure 1.

The abscissa shows time in hours. The ordinate denotes the percentage of the administered dose excreted after injection, plotted logarithmically.

TABLE 1

Patient No., Age & Sex	Diagnosis	Per cent 3:3′:5′-Th in injected dose	Per cent dose per liter serum at various times after injection				Per cent dose excreted in urine					
			5 min.	1 hr.	2 hrs.	4 hrs.	0–1 hr.	0–2 hrs.	0–4 hrs.	0–8 hrs.	0–12 hrs.	0–24 hrs.
1. 28F	No disease	88.0	36.0	9.6	5.9	—	9.1	14.9	23.0	37.5	—	—
2. 49M	Alcoholism	98.2	33.9	9.8	5.8	4.0	7.0	10.5	28.3	—	62.3	76.9
3. 35F	Alcoholism	87.8	34.7	9.5	5.5	3.4	7.1	14.1	—	—	—	—
4. 39F	Coronary heart disease	100.0	20.8**	7.2	—	—	1.4	2.6	5.3	—	18.1	35.2
5. 72F	Reticulum-cell sarcoma	88.0	30.2	7.1†	6.2	4.5	2.3†	3.7	7.8	13.3	24.9	44.8
6.* 39M	Spontaneous pneumothorax	97.7	30.5	6.6	4.1	3.2	7.3	16.3	28.2	49.7	65.2	87.0
7. 67F	Nutritional deficiency	99.0	37.9	14.5	—	—	3.6	6.4	13.5	27.1	—	—
8. 78F	Hypernephroma	90.0	—	8.3	—	—	2.8	6.2	15.4	—	—	—
9. 75F	Temporal arteritis	90.0	32.7	9.4	—	—	1.7	6.6	11.2	44.9	47.0	67.1
10. 35M	Pulmonary cyst	90.0	20.3*	4.7	—	2.1	10.8	22.8‡	42.2§	48.5	58.1	69.6

* Given 1 Gm. of KI one hour before injection of 3:3′:5′-Th.
** At 8 minutes.
† At 75 minutes.
‡ At 0 to 3 hours.
§ At 0 to 6 hours, 15 minutes.

TABLE 2. DISTRIBUTION OF I^{131}-LABELED 3:3′:5′-Th BETWEEN 3:3′:5′-Th AND I^- FRACTIONS IN THE SERUM OF 7 PATIENTS FOLLOWING INJECTION

(Values are uncorrected for small amount of I^- which was present in administered dose)

Patient	Time after injection	Per cent dose per liter serum, total	Per cent dose per liter serum, 3:3′:5′-Th fraction	Per cent dose per liter serum, I^- fraction	Per cent of sample in I^- fraction
3	5 min.	34.7	27.1	7.6	21.8
	1 hr.	9.5	1.0	8.5	90.0
	2 hrs.	5.5	0.8	4.7	86.2
	4 hr.	3.4	0	3.4	100.0
4	8 min.	20.8	18.7	2.1	10.0
	35 min.	11.5	8.5	3.0	25.9
	1 hr.	7.2	4.7	2.5	34.9
5	5 min.	30.2	23.9	6.3	20.8
	15 min.	24.9	17.8	7.1	28.6
	30 min.	13.3	9.4	3.9	29.1
	2 hrs.	6.2	3.7	2.5	40.5
	4 hrs.	4.5	2.0	2.5	55.6
6	5 min.	30.5	29.1	1.4	4.7
	15 min.	19.7	17.5	2.2	11.4
	30 min.	13.0	10.2	2.8	21.8
	1 hr.	6.6	3.7	2.9	43.4
	2 hrs.	4.1	1.3	2.8	68.3
	4 hrs.	3.2	0.6	2.6	81.6
7	5 min.	37.9	32.2	5.7	15.0
	1 hr.	14.5	9.8	4.7	32.2
8	25 min.	14.9	11.1	3.8	25.8
	1 hr.	8.3	2.7	5.6	66.9
9	5 min.	32.7	29.7	3.0	9.2
	1 hr.	9.4	4.8	4.6	49.1

of free iodide to 3:3′:5′-Th increased steadily and rapidly in successive serum samples following injection, as is shown in the last column of Table 2. No significant labeled iodide was detected except in the 3:3′:5′-Th and iodide zones of the chromatograms.

Almost all of the labeled iodine in the urine was free iodide. In a few subjects, unchanged 3:3′:5′-Th was recovered from the urine in amounts less than 6 per cent of the administered dose and was found only in the first few hours following injection. No significant labeled iodine in the urine corresponded to zones other than iodide and 3:3′:5′-Th.

Measurements of labeled iodine in the thyroid were made in 2 patients

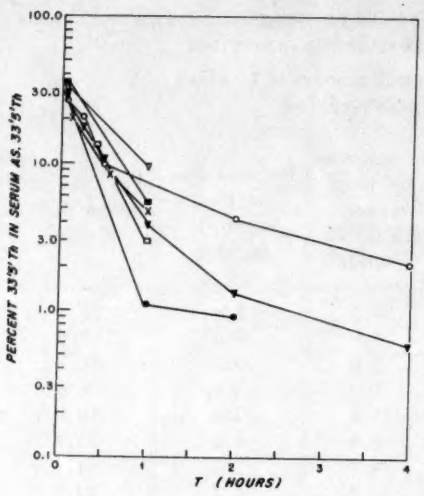

FIG. 3. Disappearance of 3:3′:5′-Th from serum in 7 patients.

The abscissa shows time in hours. The ordinate denotes the percentage of the dose of 3:3′:5′-Th per liter of serum present as unchanged 3:3′:5′-Th.

(Nos. 5 and 6), four and twenty-four hours after administration of 3:3′:5′-Th. The values were 14.6 and 7.8 per cent at four hours and 11.1 and 0 per cent at twenty-four hours. The latter patient was given 1 Gm. of KI an hour prior to the injection.

DISCUSSION

Roche and his associates have investigated the metabolism of 3:3′:5′-Th in the rat. By chromatographic separation they demonstrated I^{131}-labeled 3:3′:5′-Th in the thyroglobulin of animals forty-eight hours after injection of I^{131} (1). They also reported I^{131}-labeled 3:3′:5′-Th in the plasma twenty-four hours following administration of I^{131} (4). Tissue cultures of rat thyroids were stimulated with thyrotropin to produce 3:3′:5′-Th in significant amounts (7). Roche concluded from these studies that 3:3′:5′-Th is a naturally occurring thyroid hormone in thyroglobulin and blood.

The excretion of injected I^{131}-labeled 3:3′:5′-Th in the urine and feces of thyroidectomized rats was also investigated by Roche *et al.* (5). Approximately 27 per cent of the administered labeled iodine was recovered from the urine in the first thirty hours and an additional 3 per cent in the ensuing sixty hours. More than 90 per cent of the urinary I^{131} was in the form of free iodide; approximately 2.5 per cent was thought to be 3:3′:5′-triiodothyropyruvic acid. Twenty per cent of the injected labeled iodine was recovered in the feces. Almost all of this appeared in the first twenty-four hours and was chiefly 3:3′:5′-Th. In another experiment, 30 per cent of the injected I^{131}-labeled 3:3′:5′-Th was recovered in the first ten hours by bile-duct catheterization, principally as either the unchanged parent

compound or its glucuronic-acid conjugate. The metabolism of 3:3′:5′-Th in the rat was thought to be quite similar to that of thyroxine.

The present report indicates certain differences for the metabolism of 3:3′:5′-Th in man. As 3:3′:5′-Th disappears from the serum, labeled iodide appears. The time course of disappearance of labeled iodine after the first hour is similar to that observed after administration of I^{131}. The disposal curve of 3:3′:5′-Th was so rapid[1] and so nearly complete within the first four hours that it is not possible to assign a disposal rate with any degree of accuracy, but it is apparent from inspection of Figure 3 that 3:3′:5′-Th disappears rapidly from the blood with a half-time of the order of one-half hour. This is not attributable to a large volume of distribution, but to degradation, as shown by the recovery of iodide both in serum and urine.

The labeled iodine in the 3:3′:5′-Th used in these studies was in the 3′ and 5′ positions. If the first step in degradation is removal of iodine from the β-ring, the resulting compound would be labeled 3:3′-Th. This substance is degraded in man at a rate comparable to that of 3:3′:5′-Th, and was not detected in any of the serum or urine samples. If the initial step in degradation is removal of iodide from the α-ring, then labeled 3′:5′-Th should have been detected. No unidentified zones of labeled iodine were found on the chromatograms. It can be concluded that in man 3:3′:5′-Th is deiodinated rapidly and completely.

The physiologic role of 3:3′-Th or of 3:3′:5′-Th, if indeed there be one, remains to be shown. Their rapid degradation and disposal in man are such that there could be only minute concentrations in the plasma. In order to maintain an appreciable concentration in the blood, the secretion rate of labeled iodine from the thyroid would have to be much faster than is actually observed. It seems probable that both these substances are either unnatural in man, or if produced are, like the iodotyrosines, either degraded in the gland or used as precursors for hormone production.

Acknowledgments

3-Monoiodothyronine, 3:3′-diiodothyronine, and 3:3′:5′-triiodothyronine were generously furnished by Warner-Chilcott Laboratories.

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PHYSIOLOGIC EFFECTS OF *l*-TRIIODOTHYRONINE*

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ABSTRACT

The physiologic effects of prolonged administration of *l*-triiodothyronine (50–200 micrograms daily by mouth) were studied in a group of 10 male and 8 female institutionalized chronic psychiatric patients for thirty-eight weeks, during which 340 blood samples were obtained. All blood samples were analyzed for concentration of serum low- and high-density lipoproteins, total cholesterol and protein-bound iodine. Body weight, blood pressure, and pulse rate were recorded for each patient at the time of venipuncture. Statistical analysis of the data showed: 1) Marked lipoprotein-specific reductions in $S_f°$ 0–12 and $S_f°$ 12–20 and in total cholesterol levels in both male and female patients. Statistically significant reductions in $S_f°$ 20–100 and $S_f°$ 100–400 serum lipoproteins occurred only in female patients. 2) A distinctly positive correlation was found in female patients between the degree of reduction of $S_f°$ 0–20 serum lipoprotein concentration caused by *l*-triiodothyronine and the initial $S_f°$ 0–20 levels; in male patients this correlation was of only borderline significance and was limited to the $S_f°$ 12–20 serum lipoprotein class. A similar relationship held for total serum cholesterol levels in the female but not in the male patients. 3) Serum protein-bound iodine levels decreased markedly during administration of *l*-triiodothyronine. 4) Moderate hyperthyroid reactions were observed in 4 patients receiving 150–200 micrograms of *l*-triiodothyronine per day. The hormone did not appear to have any effect on the psychiatric status of the chronic schizophrenic patients in this study during a 9-month period of observation. 5) A quantitative equivalence was noted in the hypolipoproteinemic effect of 200 micrograms per day of *l*-triiodothyronine (orally), 5 grains per day of desiccated thyroid (orally) and 6 mg. per week of *l*-thyroxine (intravenously). 6) An apparent difference in the effects of *l*-triiodothyronine and *l*-thyroxine on the major high-density serum lipoprotein component, HDL-3, was observed. Whereas *l*-thyroxine produced a highly significant and sustained reduction in the concentration of HDL-3, no consistent hypolipoproteinemic effect could be demonstrated with *l*-triiodothyronine in the female group of patients.

IN RECENT years intensive studies of serum lipids have yielded much information about the serum lipid transport system in the human subject (1–3). Among other findings, it has been shown that both "free" and

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esterified cholesterol do not occur in the "free" state in human serum but rather in combination with a whole spectrum of lipoproteins. Different classes of serum lipoproteins, as defined by their rate of flotation in an ultracentrifugal field under standard conditions, contain varying amounts of cholesterol, ranging from 15 per cent by weight for the S_f^0 20–400 class to 33 per cent for the S_f^0 0–20 class. In view of these developments it became of interest to reinvestigate the well known hypocholesterolemic effect of thyroid hormone in order to determine if the hormone has a lipoprotein-specific action which would not be detectable by measurements of serum cholesterol concentration.

Long-term controlled studies with various dosages of desiccated thyroid (4, 5) and with *l*-thyroxine (6) have shown clearly that thyroid hormone affects predominantly the S_f^0 0–20 serum lipoproteins. After the discovery of *l*-triiodothyronine as a circulating thyroid hormone (7), numerous studies of the physiologic and biochemical effects of this substance appeared (8–19), but there are no data concerning its effect on serum lipoproteins. In order to develop such information, the present study with high dosages of *l*-triiodothyronine was undertaken.

METHODS AND CLINICAL MATERIAL

A group of 10 male and 8 female hospitalized chronic psychiatric patients, free from other disease as judged by routine laboratory tests and physical examination, were studied for a period of thirty-eight weeks. Each patient served as his own control, several blood samples being obtained before, as well as during and following administration of triiodothyronine. In addition, 2 male and 2 female patients not receiving any medication for the entire period of thirty-eight weeks were studied as a control group.

Seventeen blood samples were obtained on each patient as follows: 4 samples during a 6-week period preceding administration of *l*-triiodothyronine to serve as a baseline, 10 samples during a 23-week interval of drug administration, and 3 samples during a 9-week period following the last dose of *l*-triiodothyronine.

All blood samples were analyzed for serum lipoprotein concentrations by ultracentrifugal methods (20) and for total serum cholesterol (21) and protein-bound iodine (PBI) levels by standard chemical methods (22, 23).

Blood pressure, pulse rate, and body weight were also measured in each patient at the time of blood sampling. All patients received a daily oral dose of *l*-triiodothyronine between 7–8 a.m. as follows:

50 micrograms for a 2-week period, followed by
100 micrograms for a 7-week period, followed by
150 micrograms for a 2-week period, followed by
200 micrograms for an 11-week period.

Two additional male patients did not complete this schedule because of intercurrent surgical conditions (acute inguinal hernia, and infected varicose vein ulcers on both lower extremities) necessitating transfer to other wards. Because of the unknown effect of acute illness and surgery on serum lipoprotein levels, and of difficulties in adequate supervision of the thyroid hormone dosage schedule, these 2 patients were excluded from

the study. In 1 female patient of small body stature and weighing 90–95 pounds, the 150-microgram regimen of *l*-triiodothyronine was continued for the 11-week period during which all other patients received 200 micrograms daily.

RESULTS

The effects of *l*-triiodothyronine on serum lipoprotein, total cholesterol and PBI levels, and on blood pressure, pulse rate and body weight are shown in Figures 1–4. Each graph represents the time-course of the measured variable expressed as the mean value for the male or female patient group.

Effect of l-triiodothyronine on serum lipoprotein and total cholesterol levels

It may be seen from these graphs that *l*-triiodothyronine caused marked reductions in the levels of $S_f°$ 0–12 serum lipoprotein and total cholesterol in both male and female patients. However, increasing differences between the male and female groups in the response to *l*-triiodothyronine were evident in the $S_f°$ 12–20, $S_f°$ 20–100, and $S_f°$ 100–400 serum lipoprotein classes. Thus, whereas mean $S_f°$ 12–20 serum lipoprotein values decreased only slightly in the male group (significant at the 5 per cent level), sizable reductions in these mean levels occurred in the female group (significant at the 0.1 per cent level). Furthermore, no significant changes in $S_f°$ 20–100 and $S_f°$ 100–400 serum lipoprotein levels occurred in the male group, whereas small but definite reductions were noted in the female group (significant at the 1 per cent and 2 per cent levels, respectively).

The only substantial effect of *l*-triiodothyronine on the level of high-density serum lipoproteins was limited to the HDL-2 class, which was reduced by 25 per cent in the female group of patients receiving 150–200 micrograms of the hormone (significant at the 1 per cent level). No corresponding effect occurred in the male group. A slight decrease in HDL-3 serum lipoprotein concentration, of borderline statistical significance, was also observed in the male but not in the female group.

Table 1 presents a summary of the effect of *l*-triiodothyronine on the levels of serum lipids and cholesterol and of the statistical significance of these changes.

Effect of l-triiodothyronine on blood pressures, pulse rate, body weight and serum protein-bound iodine level

Moderate, but statistically significant changes were observed in mean blood pressure, pulse rate, and body weight in both the male and female groups (Table 2). Systolic and diastolic blood pressure decreased, the pulse rate increased, and body weight decreased.

In general these changes were greater at the higher *l*-triiodothyronine

dosage levels (150–200 micrograms); perhaps the only changes sufficiently large to be of possible clinical, as opposed to statistical significance, were noted at the 150–200 microgram dosage levels and occurred in mean diastolic blood pressure (lowered by 10–15 mm. Hg), pulse rate (increased by 9 beats per minute), and body weight (lowered by 5–8 pounds in the male

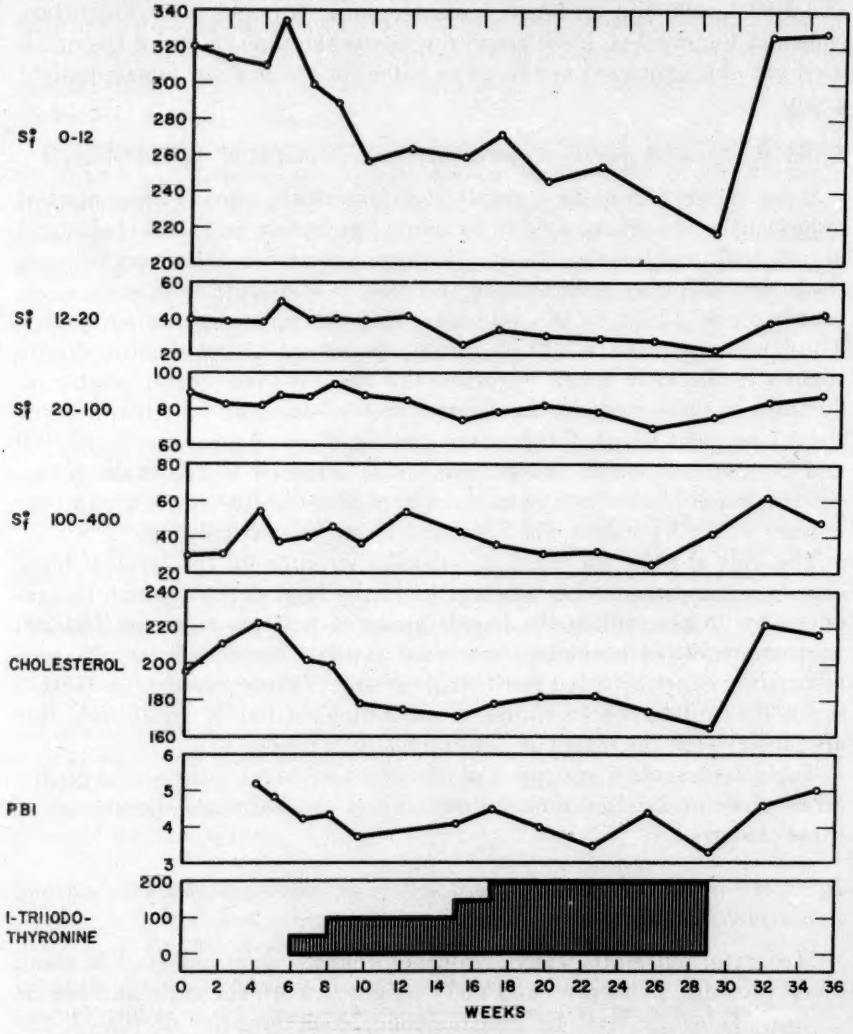

FIG. 1. The effect of *l*-triiodothyronine (μg./day) on mean serum lipoprotein (mg./100 ml.), total cholesterol (mg./100 ml.), and protein-bound iodine levels (μg./100 ml.) in a group of 10 male patients hospitalized for chronic psychiatric disorders.

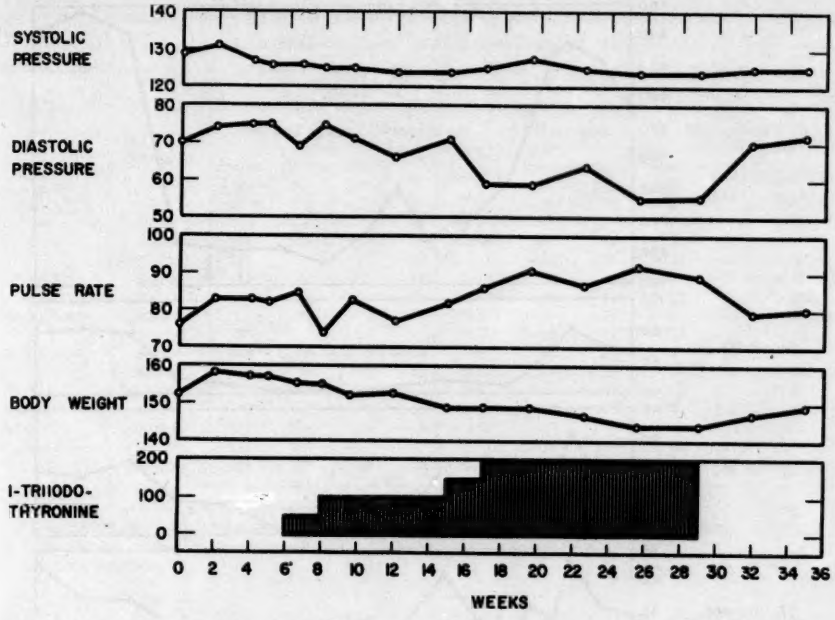

FIG. 2. The effect of *l*-triiodothyronine (μg./day) on mean blood pressure (mm. Hg), pulse rate (beats/min.), and body weight (pounds) in a group of 10 male patients hospitalized for chronic psychiatric disorders.

group). Except for the greater drop in systolic blood pressure and the slightly smaller weight loss observed in the female group during the 150–200 microgram dosage of *l*-triiodothyronine, no consistent difference between the male and female group was noted.

There was a downward trend of mean serum PBI levels during the period of triiodothyronine administration, becoming more definite at dosage levels of 150–200 micrograms. Figures 1 and 3 and statistical tests showed a slightly greater reduction in mean PBI levels in the female group (significant at the 1 per cent level) than in the male group (significant at the 5 per cent level) (Table 2).

DISCUSSION

Incidence of side-reactions

Difficulties arise in the selection of criteria by which to define side-reactions or hyperthyroid reactions. Descriptions of subjective feelings obviously are of little value in a psychotic group of patients, but even objective criteria such as increased nervousness, hyperactivity, tremor or agitation are difficult to employ in a population showing some of these criteria to a variable degree during the pretreatment "baseline" period. Pulse rate and

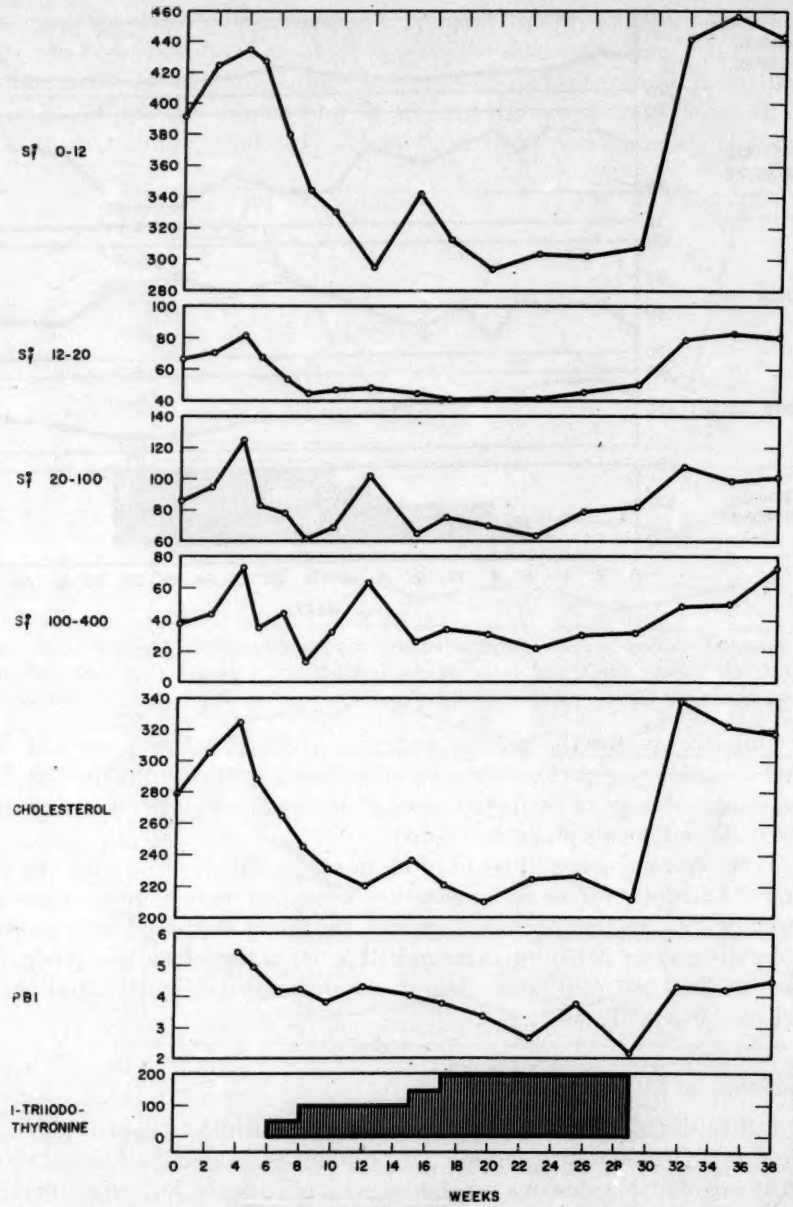

FIG. 3. The effect of *l*-triiodothyronine (μg./day) on mean serum lipoprotein (mg./100 ml.), total cholesterol (mg./100 ml.) and protein-bound iodine levels (μg./100 ml.) in a group of 8 female patients hospitalized for chronic psychiatric disorders.

TABLE 1. EFFECT OF *l*-TRIIODOTHYRONINE ON SERUM LIPOPROTEIN AND TOTAL CHOLESTEROL CONCENTRATIONS

	Tri-Th, daily dose (μg.)	Male group					Female group				
		Mean levels		Mean change			Mean levels		Mean change		
		Before & after	During Tri-Th	Mg./100 ml.	Per cent	Signif. P value	Before & after	During Tri-Th	Mg./100 ml.	Per cent	Signif. P value
$S_f°$ 0–12*	50–200†	318	260	−58	−18	<0.001	431	321	−110	−25	<0.001
	150–200‡	318	245	−73	−23	<0.001	431	304	−127	−29	<0.001
$S_f°$ 12–20	50–200	41	34	− 7	−17	<0.05	76	46	− 30	−39	<0.001
	150–200	41	28	−13	−32	<0.001	76	44	− 32	−42	<0.001
$S_f°$ 20–100	50–200	85	82	− 3	− 3	N.S.§	99	74	− 25	−25	<0.005
	150–200	85	77	− 8	− 9	<0.02	99	72	− 27	−27	<0.01
$S_f°$ 100–400	50–200	43	38	− 6	−14	N.S.	51	33	− 18	−35	<0.02
	150–200	43	33	−10	−23	N.S.	51	29	− 22	−43	<0.02
HDL-1	50–200	20	19	− 1	− 5	N.S.	22	24	+ 2	+10	N.S.
HDL-2	50–200	53	47	− 6	−11	N.S.	133	100	− 33	−25	≃0.01
HDL-3	50–200	212	196	−16	− 8	<0.05	263	260	− 3	− 1	N.S.
Cholesterol	50–200	218	181	−37	−17	<0.001	311	229	− 82	−26	<0.001
	150–200	218	177	−41	−19	<0.001	311	219	− 92	−29	<0.001

* All serum lipoprotein and cholesterol concentrations are expressed as mg./100 ml. of serum.
† The 50–200 μg. dosage level refers to the entire 23-week period of triiodothyronine administration.
‡ The 150–200 μg. dosage level refers only to this 13-week period of triiodothyronine administration.
§ Not significant.

blood pressure may be more labile in a psychotic population (24) and the measurements are therefore of limited value as indicators of hyperthyroid reactions. As there is no completely satisfactory solution of these difficulties, the clinical impressions together with all available measured parameters were used as criteria of side-reactions.

TABLE 2. EFFECT OF *l*-TRIIODOTHYRONINE ON BLOOD PRESSURE, PULSE RATE, BODY WEIGHT AND SERUM PROTEIN-BOUND IODINE LEVEL

	Tri-Th, daily dose (μg.)	Male group					Female group				
		Mean levels		Mean change			Mean levels		Mean change		
		Before & after	During Tri-Th	Mg./100 ml.	Per cent	Signif. P value	Before & after	During Tri-Th	Mg./100 ml.	Per cent	Signif. P value
Syst.*	50–200	127	125	− 2	− 2	<0.05	131	122	− 9	− 7	<0.01
B.P.	150–200	127	125	− 2	− 2	N.S.	131	122	− 9	− 7	<0.05
Diast.	50–200	73	64	− 9	−12	<0.02	80	72	− 8	−10	<0.001
B.P.	150–200	73	58	−15	−20	<0.001	80	69	−11	−14	<0.001
Pulse	50–200	81	85	+ 4	+ 5	N.S.	95	102	+ 7	+ 7	<0.05
rate	150–200	81	90	+ 9	+11	<0.001	95	104	+ 9	+ 9	≃0.02
Body wt.	50–200	155	150	− 5	− 3	<0.02	130	126	− 4	− 3	<0.001
	150–200	155	147	− 8	− 5	<0.001	130	125	− 5	− 4	<0.001
Serum	50–200	4.9	4.0	− 0.9	−18	<0.01	4.7	3.6	− 1.1	−23	≃0.02
PBI	150–200	4.9	3.8	− 1.1	−22	<0.05	4.7	3.0	− 1.7	−36	<0.01

* Blood pressure in mm. of mercury; pulse rate in beats/minute; body weight in pounds; and serum PBI level in μg./100 ml.

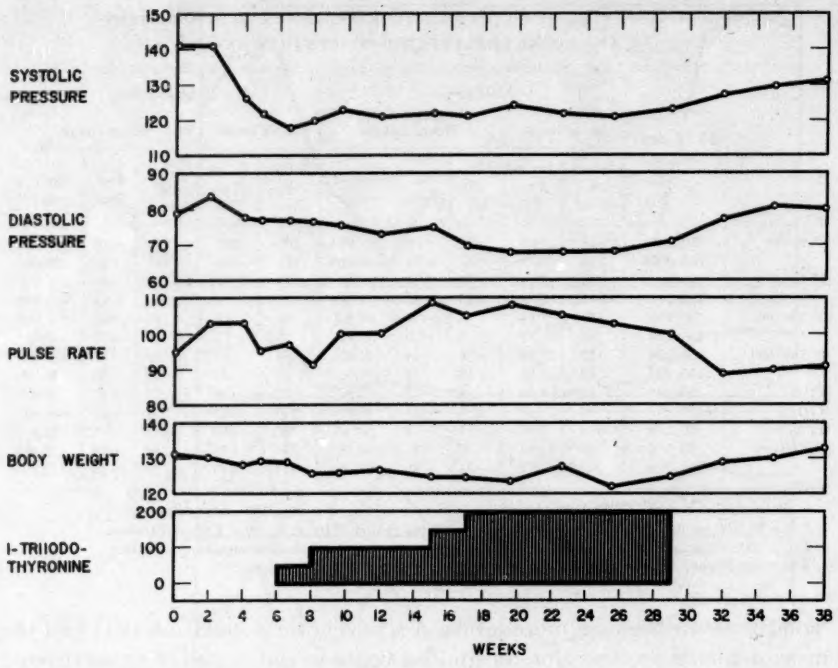

FIG. 4. The effect of *l*-triiodothyronine (μg./day) on mean blood pressure (mm. Hg), pulse rate (beats/min.), and body weight (pounds) in a group of 8 female patients hospitalized for chronic psychiatric disorders.

Moderate to marked signs of hyperthyroidism were noted in 3 male and in 1 female patient. The manifestations of hyperthyroidism consisted of excessive weight loss (2–3 times greater than the mean for the group), hyperactivity, tremors, episodes of transient flushing of the face, and brief bouts of "weakness."

The absence of overt hyperthyroid reactions during administration of *l*-triiodothyronine at a dosage level of 100 μg. per day should be noted. Because of the lack of biologic activity of the dextrorotatory isomer (9, 25), this amount is approximately biologically equivalent to 200 micrograms of *dl*-triiodothyronine daily—a dosage which is at the upper end of the range heretofore used in human subjects for more than very short periods of time.

Table 3 presents a summary of the incidence of hyperthyroid reactions in the present study, compared with that in previous similar studies with desiccated thyroid (4, 5) and with *l*-thyroxine (6). It may be seen that in these 3 groups the incidence of hyperthyroid reactions varied from 6 to 22

TABLE 3. INCIDENCE OF HYPERTHYROID REACTIONS IN HOSPITALIZED CHRONIC PSYCHIATRIC PATIENTS DURING TREATMENT WITH *l*-TRIIODOTHYRONINE *vs.* *l*-THYROXINE AND DESICCATED THYROID

Hyperthyroid reaction	*l*-Triiodothyronine, 50–200 μg./day, orally, 23 wks.—Hosp. A.	*l*-Thyroxine, 6 mg./wk., i.v., 16 wks.—Hosp. A.	Desiccated thyroid, 3–4 gr./day, orally, 72 wks.—Hosp. B.
Incidence	In 4* of 18 patients (22%)	In 1 of 16 patients (6%)	In 6 of 60 patients (10%)
Manifestation	Hyperactivity and incr. nervousness (4 pats.)		Hyperactivity (2 pats.)
	Excessive wt. loss also (2 of above 4 pats.)	Wt. loss (1 pat.)	Excessive wt. loss (4 pats.)

* No instance of hyperthyroid reaction was observed at dosage levels below 150 μg./day.

per cent, being highest with *l*-triiodothyronine, and lowest with *l*-thyroxine. However, the significance of these differences is somewhat uncertain, since they are based on a total of only 11 cases of hyperthyroid reactions observed in analogous studies on patients in 2 separate state mental institutions (Hospitals A and B, Table 3).

Effect of l-triiodothyronine on psychiatric status

Although the present study was primarily undertaken to study the hypolipoproteinemic effect of *l*-triiodothyronine, the interesting possibility of an effect of this hormone on the psychiatric status of each patient was considered and continuous general observations for any change in mental status were made by one of us (E.H.S.) and by staff psychiatrists.

No instance of definite improvement or deterioration in mental status was noted, though some patients began to cooperate somewhat more closely with us. However, it was felt that this change in attitude was entirely attributable to a positive, helping and friendly attitude and prolonged contact of the investigating physicians with the patients. It is of interest that even the most cooperative patients retained their psychotic symptoms such as paranoid delusions, ideas of reference, bizarre compulsive behavior patterns, complete refusal to talk, mechanistic repetitive behavior, or moods of depression or of inappropriate elation.

The experience gained in the present study extending over a period of nine months does not support observations recently reported by Flach and associates (26) regarding significant improvement of psychotic symptoms in a group of patients receiving triiodothyronine. The reasons for these divergent observations are not clear at this time, though special selection of

patients may be a factor. All the subjects in our group were chronic psychotic patients who were euthyroid as judged by clinical criteria and the serum PBI level.

Relationship between initial serum lipoprotein levels and the hypolipoproteinemic effect of l-triiodothyronine

Of clinical significance in the use of *l*-triiodothyronine is the possible relationship between initial serum lipid levels and the change in these levels which may be expected to result from use of this hormone. To answer this question a rank-correlation coefficient between the initial (pre-treatment) serum lipid level and change in this level was calculated for each serum lipoprotein band and for total serum cholesterol concentration for both male and female groups.

Table 4 presents the results of this calculation, including P values for the significance of the rank-correlation coefficients. This table shows that: 1) in the male group only the reduction observed in the $S_f°$ 12–20 serum

TABLE 4. RELATION OF INITIAL (PRE-TREATMENT) SERUM LIPOPROTEIN AND CHOLESTEROL CONCENTRATIONS TO THE CHANGE IN THESE CONCENTRATIONS PRODUCED BY *l*-TRIIODOTHYRONINE

	Range in 10 Male Patients				Range in 8 Female Patients			
	Initial levels (mg./100 ml.)	Change in levels	RCC*	Signif. of RCC (P value)	Initial levels (mg./100 ml.)	Change in levels	RCC*	Signif. of RCC (P value)
Cholesterol	164–286	−15 to − 51	0.12	N.S.†	233–359	− 37 to −124	0.83	≃0.01
$S_f°$ 0–12	213–369	−15 to −125	0.06	N.S.	322–503	− 55 to −155	0.83	≃0.01
$S_f°$ 12–20	25– 91	0 to − 18	0.60	<0.05	36–111	− 2 to − 55	0.85	<0.01
$S_f°$ 20–100	41–139	−16 to + 17	0.43	N.S.	32–185	− 88 to + 10	0.36	N.S.
$S_f°$ 100–400	7–159	− 8 to + 13	0.17	N.S.	6–201	−133 to + 42	0.39	N.S.

* Rank-Correlation Coefficient.
† Not significant.

lipoprotein concentrations was significantly correlated with initial pre-treatment levels, whereas the reductions observed in all remaining serum lipoprotein classes and in the serum cholesterol level did not appear to be significantly related to the corresponding initial levels, at least within the limited ranges studied in this investigation; 2) in the female group the reductions in $S_f°$ 0–12 and $S_f°$ 12–20 serum lipoprotein concentrations and in the serum total cholesterol concentration were significantly related to the corresponding initial levels, showing that greater reductions occurred in the higher lipid concentration ranges; and 3) no statistically significant correlation coefficients between initial levels and change in levels produced by *l*-triiodothyronine could be established for the $S_f°$ 20–100 and $S_f°$ 100–400 serum lipoprotein classes in either the male or female group.

The reason for these differences in response to *l*-triiodothyronine between male and female patients is not clear at this time. A partial explanation may lie in the different distribution of the initial lipid ranges (lower in the male group), or in the more adequate and reliable supervision of the female group by the ward personnel with regard to actual ingestion of the medication on a day-to-day basis.

The level of serum protein-bound iodine

A marked lowering of the serum PBI level occurred following ingestion of large doses of *l*-triiodothyronine. Similar observations were made by Starr *et al.* in a case of hypopituitary hypothyroidism (11) and in healthy normal human volunteers who received *l*-triiodothyronine in daily doses of 4–140 micrograms for a period of one week (27).

In the present study a significant decrease in the serum PBI level occurred in each of 8 female and in 7 of 10 male patients at dosage levels of 150–200 micrograms of *l*-triiodothyronine daily. The decreases ranged from 0.8 to 4.2 μg. per 100 ml., with a mean decrease of 2.1 μg. per 100 ml.

Whereas both thyroxine and *l*-triiodothyronine cause a reduction in thyroidal I^{131} uptake (27–29), thyroxine produces an *increase* in the serum PBI level (30). Since it has been shown that both hormones combine readily with certain plasma proteins *in vitro* (31–34) and *in vivo* (33), at least a transient rise in serum PBI concentration may be expected following administration of thyroxine. It has been reported (30) that daily ingestion by healthy young adults of 0.6 mg. of *l*-thyroxine for nine days produced a continuous rise in the serum PBI levels, reaching 15–16 μg. per 100 ml. The PBI levels decreased promptly after administration of thyroxine was stopped, and they returned to the euthyroid range ten days later. Evidently the elevation in PBI concentration resulted largely from distribution of ingested thyroxine over the extrathyroidal organic iodine space.

It is well established that the release of thyrotropin by the pituitary is inhibited by the administration of thyroxine or *l*-triiodothyronine (38–42). In addition to this, 3 factors may be listed which tend to decrease serum PBI levels following administration of *l*-triiodothyronine: 1) less *l*-triiodothyronine is required, relative to the amount of thyroxine, to produce a given physiologic effect, 2) the distribution of *l*-triiodothyronine is probably over a larger volume compared to that of thyroxine (35), and 3) the more rapid deiodinization of triiodothyronine (36–37).

Comparison of the physiologic effects of triiodothyronine, thyroxine and desiccated thyroid

Since both *l*-thyroxine and *l*-triiodothyronine have qualitatively similar physiologic and biochemical effects, it is noteworthy that changes in the

PBI level were not necessarily proportional to the metabolic and physiologic effects produced by thyroid hormones.

It is of clinical and scientific interest to observe the relationship between the physiologic effects of *l*-triiodothyronine and those of other thyroid hormones. Table 5 presents such a comparison. The data relating to *l*-triiodothyronine were obtained in the present study, whereas the remaining data

TABLE 5. COMPARISON OF THE EFFECTS OF *l*-TRIIODOTHYRONINE, *l*-THYROXINE AND DESICCATED THYROID

	l-Triiodothyronine (200 μg./day, orally, for 11 wks.)			*l*-Thyroxine (6 mg./wk., i.v., for 16 wks.)			Desiccated thyroid (5 grs./day, orally, for 39 wks.)		
	Initial value	Change	Per cent change	Initial value	Change	Per cent change	Initial value	Change	Per cent change
$S_f°$ 0– 20†	419	−130	−31	417	−145	−35	432	−107	−25
$S_f°$ 20–400	132	− 27	−20	125	− 11	− 9	122	− 27	−22
$S_f°$ 0–400	551	−157	−29	542	−156	−29	554	−134	−24
HDL-1 (M&F)	21	+ 1	+ 5	23	− 4	−17			
HDL-2 (M)	53	− 6	−11	58	− 22	−38	Data not available on HDL concentrations.		
HDL-2 (F)	133	− 33	−25	69	− 12	−17			
HDL-3 (M)	212	− 16	− 8	206	− 37	−18			
HDL-3 (F)	263	− 3	− 1	233	− 35	−15			
Body weight (lbs.)	144	− 7	− 5	146	− 13*	− 9	145	− 5*	− 3
Systolic B.P. (mm.Hg)	130	− 6**	− 5	125	0	0	112	± 8**	+ 7
Diastolic B.P.	76	− 13**	−17	78	− 11	−14	73	0**	0
Pulse rate (beats/min.)	89	+ 7	+ 8	81	+ 16**	+20	84	+ 6**	+ 7
No. of patients	18 (10M&8F)			13 (7M&6F)			39 (20M& 19F)		

† All serum lipoprotein values are in terms of mg./100 ml.
* $P < 0.05$ to $P < 0.02$.
** $P < 0.001$ to $P < 0.01$.
The P values refer to the statistical significance of the *difference* between starred figures in any given row.

were based on analogous long-term studies with desiccated thyroid (4, 5) and *l*-thyroxine (6).

To facilitate discussion, these data are considered under 3 headings: 1) the effect of the 3 thyroid-active substances on low-density serum lipoprotein concentrations; 2) their effect on high-density serum lipoprotein concentrations; and 3) their effect on blood pressure, pulse rate and body weight.

The levels of low-density serum lipoprotein ($S_f°$ 0–400) were lowered by 24–29 per cent by each of the 3 thyroid-active substances, with 80–93 per cent of the total decrease being due to reduction of the concentration of the $S_f°$ 0–20 band. The remarkable uniformity of this hypolipoproteinemic effect suggests that dose equivalents can be estimated, at least with respect to this one criterion. Ingestion of 200 micrograms per day of *l*-triiodothyronine appears equivalent to an oral intake of 5 grains of desiccated thyroid per day, and each is equivalent to 6 mg. of *l*-thyroxine given intrave-

nously per week. Thus on a weight basis, *l*-triiodothyronine appears to be 4.3 times more potent than *l*-thyroxine, and about 1,620 times more active than desiccated thyroid. These observations are in agreement with those of other workers who have reported *l*-triiodothyronine to be 3 to 5 times more effective, on a molar basis, in the treatment of myxedema (11, 13, 42).

The data on high-density serum lipoprotein levels show that neither *l*-triiodothyronine nor *l*-thyroxine affect the concentration of HDL-1 serum lipoprotein to any significant extent. *l*-Thyroxine has a somewhat greater hypolipoproteinemic effect on the HDL-2 class ($P < 0.01$) than does *l*-triiodothyronine (P not significant in the male group, though $P = 0.01$ in the female group).

Table 5 reveals a possible difference in the effects of *l*-triiodothyronine and *l*-thyroxine on HDL-3, the most abundant of the high-density serum lipoproteins. *l*-Triiodothyronine appeared to have only a small or no hypolipoproteinemic effect, particularly in the female group of patients ($P < 0.05$ for the males; P not significant for the females), whereas *l*-thyroxine produced a definite and consistent lowering of HDL-3 serum lipoprotein levels ($P < 0.001$ for both male and female patients). Studies are currently in progress to investigate in more detail this unexpected lack of hypolipoproteinemic effect of *l*-triiodothyronine.

Statistically significant differences ($P < 0.01$ to $P < 0.001$) were observed in the effects of the 3 thyroid hormones on systolic and diastolic blood pressure (lowered by *l*-triiodothyronine, and raised or unchanged by *l*-thyroxine) and in pulse rate (increased more by *l*-thyroxine than by *l*-triiodothyronine or desiccated thyroid). Weight changes of borderline significance were also noted, weight loss being greater with *l*-thyroxine than with either *l*-triiodothyronine or desiccated thyroid.

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ASSESSMENT OF THE FUNCTIONAL CAPACITY OF THE ADRENAL CORTEX. I. ESTABLISHMENT OF NORMAL VALUES*

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ABSTRACT

The functional capacity of the human adrenal cortex was studied by estimating urinary 17-hydroxycorticosteroids, total 17-ketosteroids, dehydroepiandrosterone, androsterone+etiocholanolone, and 11-oxygenated 17-ketosteroids, before and after the intravenous infusion of 25 I.U. of ACTH. The number of circulating eosinophils was also followed. Data are presented on the overall reliability (in terms of precision, accuracy, sensitivity and specificity) of the methods employed.

In all steroids or steroid groups studied, there was a highly significant correlation between the logarithms of resting and stimulation levels. Neglect of this correlation may result in an erroneous assessment of adrenocortical reserve. By far the most useful index of adrenocortical reserve capacity was found to be the estimation of urinary 17-hydroxycorticosteroids. Normal values obtained in 52 subjects and fiducial limits (within which 95 per cent of normal responses to intravenous ACTH can be expected to fall) are also presented. The use of this technique seems to provide a suitable means also for the laboratory diagnosis of latent adrenocortical hypofunction. The estimation of circulating eosinophils was found to be of distinctly limited value.

IT IS generally agreed that the response to adrenocorticotropin (ACTH) is a highly specific and important test of adrenocortical reserve (1, 2). The most frequently employed criteria for assessment of this adrenal reaction are the eosinopenic response (1), the excretion of urinary 17-ketosteroids (1), the blood level of Porter-Silber chromogens[1] (4), and the urinary excretion of butanol-extractable Porter-Silber chromogens[1] (2) or of pregnanediol (5).

It is also well established that the usefulness of either the eosinopenic

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[1] The term, "Porter-Silber chromogens" is used to denote all substances producing the characteristic color reaction of Porter and Silber (3).

or the 17-ketosteroid response is much more limited than that of methods based on the estimation of Porter-Silber chromogens in the blood or urine. The last mentioned methods provide a useful tool for the evaluation of adrenal response, but their use does not always allow of a quantitative assessment of adrenal reserve capacity. Hence the laboratory diagnosis of adrenal hypofunction often presents great difficulties.

The purpose of this communication is to present an attempt at the quantitative and qualitative assessment of the functional capacity of the adrenal cortex. The method is based on the estimation of urinary 17-hydroxycorticosteroids before and after intravenous infusion of ACTH. Furthermore, since a separate study of individual steroids, or specific steroid groups might offer improved means for a more refined diagnosis of adrenocortical dysfunction, the present study was also extended to include the estimation of urinary total 17-ketosteroids, dehydroepiandrosterone, androsterone+etiocholanolone and 11-oxygenated 17-ketosteroids.

PATIENTS

The clinical material consisted of 52 patients in the wards of the Department of Medicine, Karolinska sjukhuset, during the years 1955–1957. There were 26 males and 26 females in the age bracket of 20 to 60 years. The distribution of the sexes below and above the age of 35 was uniform. These patients were admitted for observation because of diffuse symptoms. Clinical examination did not reveal any definite somatic, endocrine or psychiatric disturbances. A few cases of congenital heart disease with negligible hemodynamic disturbances were also included.

METHODS

Definitions and abbreviations

The term *"corticosteroid"* (CS) will be used to denote all C_{21} steroids possessing at least one of the following characteristics: an α-ketol or glycol side-chain, an 11-oxygen function and/or a 17-hydroxyl group.

The term *"17-hydroxycorticosteroid"* (17-OHCS) will be used to indicate all corticosteroids possessing a 17-hydroxyl group, including compounds with the 17:20-glycol, 17,20-ketol and 17:20:21-triol as well as the dihydroxyacetone side-chain.

The term *"17-ketogenic steroid"* (17-KGS) will be used to denote all corticosteroids yielding 17-ketosteroids upon oxidation with sodium bismuthate without any pretreatment of the urine (*e.g.*, reduction by KBH_4). An estimate of 17-KGS is the difference between the value for 17-ketosteroids formed from corticosteroids upon oxidation and hydrolysis of conjugates, and the value for 17-ketosteroids liberated by hydrolysis alone.

The term *17-ketosteroid* (17-KS) will denote substances producing the characteristic color reaction with the *m*-dinitrobenzene reagent of Zimmermann (6).

The abbreviation *DHEA* will indicate dehydroepiandrosterone; *A+E* will be used to denote the sum of androsterone+etiocholanolone; and 11-oxygenated 17-ketosteroids will be abbreviated as *11-O-17-KS*.

The term *reliability* when applied to a laboratory method will be used in a general sense to indicate the 4 concepts—precision, accuracy, sensitivity and specificity. The definition and mode of assessment of these concepts have been described elsewhere (7–9).

Statistical

The standard deviation (S.D.) between duplicate estimates was calculated as suggested by Snedecor (10),

$$\text{S.D.} = \sqrt{\frac{S(d^2)}{2n}} \qquad \text{I}$$

where d is the difference between duplicates and n is the number of duplicate analyses. Implicit in the use of this formula is that the estimate should be based on a large number of replications (at least 30, or more).

Standard deviation of a ratio: a/b was calculated by use of the formula

$$s_{a/b} = \frac{\bar{a}}{\bar{b}} \sqrt{\frac{(s_a)^2}{\bar{a}^2} + \frac{(s_b)^2}{\bar{b}^2} - \frac{2s_{ab}}{n-1}} \qquad \text{II}$$

where $\bar{a}$ and $\bar{b}$ are the mean values calculated from a series of n values of a and b; $(s_a)^2$ and $(s_b)^2$ are the variances of a and b and s_{ab} denotes the sum of products of deviations, *i.e.*, $S(a-\bar{a})\cdot(b-\bar{b})$ (see, for instance, Yates (11)).

Regression lines were calculated by classical methods (see, for instance, Snedecor (12)), using a logarithmic transformation of both resting and stimulation values. In all equations the symbol x denotes resting levels, and y denotes stimulation values.

The standard error (s_b) *of the regression coefficient* (b) was calculated as

$$s_b = \sqrt{\frac{s_{yy} - \frac{(S_{xy})^2}{S_{xx}}}{(n-2)S_{xx}}} \qquad \text{III}$$

where S_{xx} and S_{yy} denote the sums of squares of deviations of x and y, respectively, *i.e.*, $S(x-\bar{x})^2$ and $S(y-\bar{y})^2$; S_{xy} is the sum of products of deviations, *i.e.*, $S(x-\bar{x})(y-\bar{y})$; and n is the number of tests.

Fiducial intervals around the regression lines, embracing the area within which 95 per cent of individual ACTH-test results are expected to fall, were calculated by standard methods (see, for instance, Goulden (13)), using the formula

$$Y_L = t\sqrt{\frac{S_{yy} - \frac{(S_{xy})^2}{S_{xx}}}{n-2} \cdot \left(1 + \frac{1}{n} + \frac{(x-\bar{x})^2}{S_{xx}}\right)} \qquad \text{IV}$$

In this formula the symbols retain the same significance as in formula III; t is the 0.05 probability deviate with $n-1$ degrees of freedom, and x any particular value (log resting level) for which the limits are sought around the regression line. It is realized that—due to the error inherent in the estimation of the independent variate—these limits are only approximate.

Estimation of 17-hydroxycorticosteroids

The present method has been evolved from the original method of Norymberski (14, 15), from our modification thereof (16), and from the recently introduced pre-reduction step of Appleby *et al.* (17). In contrast to the method of Porter and Silber (3), which measures only corticosteroids

with a dihydroxyacetone side-chain, this procedure estimates all corticosteroids possessing a 17-hydroxyl group, including thus 17,20,21-triols, 17,20-glycols, 17,20-ketols and 17,21-diol-20-ones. Continued studies of some variables affecting the estimations, such as time, temperature and pH at the reduction step (18), quality of sodium bismuthate (19), interaction between sodium bismuthate and dehydroepiandrosterone (20), and concentration of acid and time of hydrolysis (18), led to additional modifications. Therefore a brief description of the method in its present form seems to be justified.

Performance of assay

A preliminary check is made of the absence of glucose in the 24-hour collection of urine. To each of two 8-ml. aliquots of the urine is added 0.2 ml. of freshly prepared KBH_4 solution (20 per cent *w/v*). The mixture is shaken and left at room temperature for at least one hour. Then 8.0 ml. of glacial acetic acid and 3.0 Gm. of $NaBiO_3$ are added and the mixture is shaken mechanically in the dark for two hours. The contents of the tubes are centrifuged and from the supernatant liquid a 2.5-ml. aliquot is transferred into another tube. Three drops of a 5 per cent $Na_2S_2O_5$ solution (*w/v*) are added, and after stirring with a glass rod, the sample is diluted with 2.5 ml. of distilled water. Following the addition of 0.5 ml. of concentrated HCl, the rack of tubes is placed in a boiling water bath for fifteen minutes exactly. The time and temperature of hydrolysis are critical. It is therefore essential to standardize rigorously the conditions of hydrolysis, *i.e.*, always a constant number of tubes should be used. After cooling in cold water, an 8-ml. portion of freshly distilled ether is added to each tube and the tubes are shaken for three minutes. The urine is removed by suction and the ether is washed with 2.0 ml. of water and 2.5 ml. of 3 *N* NaOH. The ether extract is shaken with NaOH pellets (approximately 30) and the ether is filtered into test tubes. The ether is evaporated at 45° C. on a water bath and the *dry* residue is dissolved in a freshly prepared mixture of 0.2 ml. of 2 per cent *m*-dinitrobenzene and 0.4 ml. of 1.25 *N* KOH in absolute ethanol stabilized with ascorbic acid (21). After incubation in the dark at 25° C. for sixty minutes, the samples are diluted with 3.4 ml. of absolute ethanol and read in a Beckman spectrophotometer against a reagent blank at 460, 520 and 580 mμ. The results are calculated by using the color correction equation of Allen (22). With each series of determinations, 2 standards of approximately 30 μg. of DHEA are run in such a way that the standards form the first and last tubes of the series. The results are expressed in mg. of DHEA—equivalent per twenty-four hours.

In case the urine contains more than 0.25 per cent glucose, in order to obtain valid estimates it is advisable to extract the corticosteroids in conjugated form prior to reduction and oxidation. The method described by Edwards *et al.* (23) was found to be suitable for this purpose. In our laboratories the extraction is carried out as follows: A 15-ml. aliquot of urine is diluted with distilled water to 30 ml. Ammonium sulphate (15 Gm.) is dissolved in the diluted urine, and the solution is extracted four times with 25 ml. of an ethanol-ether mixture (1:3). The ethanol-ether phases are combined and evaporated to dryness under reduced pressure. The dry residue is dissolved in 15 ml. of a 0.2 per cent urea solution. An 8-ml. portion of this urea solution (corresponding to 8 ml. of original urine) is then reduced and oxidized as described previously. A detailed study of the variables affecting this procedure will be published elsewhere (Plantin, to be published).

Comparison between the 17-OHCS and 17-KGS-methods

In order to assess the present method, it was compared with the one previously used (16) in 100 parallel assays, both carried out in duplicates. The results of this comparison are shown in Figure 1.

Inspection of the data in this figure reveals an excellent correlation between the values obtained by the 2 methods. From the equation of the line ($Y = 0.96 + 0.728\ x$) it is also apparent that the new method yields approximately 25 per cent higher values than the former one. This difference is assumed to depend on the facts that an additional type of corticosteroid (*i.e.*, 17,20-ketols) is estimated and that the deleterious effect of $NaBiO_3$ on urinary DHEA is eliminated (20).

Reliability of the proposed method

Specificity. This was tested by infrared spectrography of the various 17-KS fractions following Girard separation and chromatography. (The method has been reported in detail (24).) The main products isolated and identified were: 3α-hydroxy-etiocholan-17-one; 3α-hydroxy-9-etiocholen-17-one; and 3α-hydroxy-etiocholane-11,17-dione. The second steroid is an artifact produced by the acid hydrolysis of 3α,11β-dihydroxy-etiocholan-17-one. Under the conditions used in the present study this transformation is

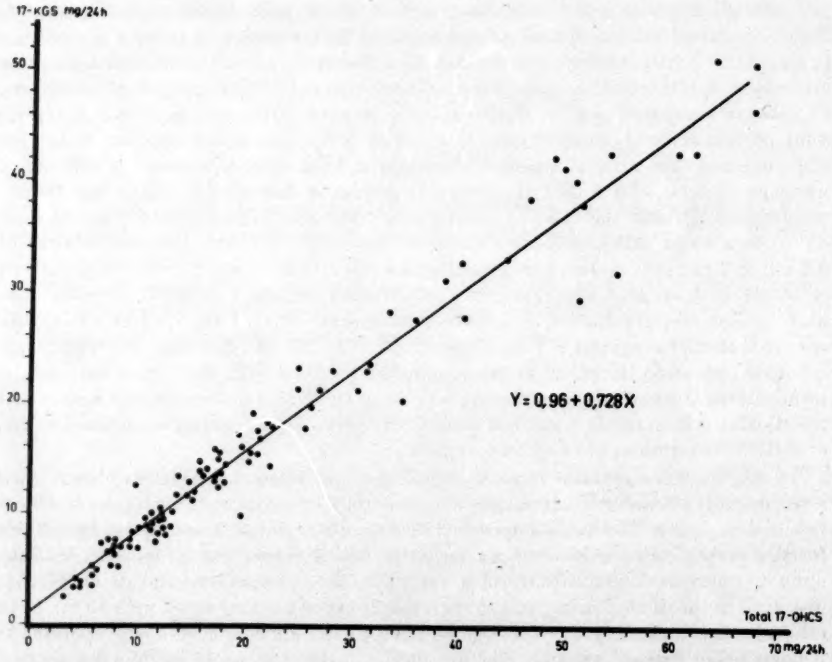

FIG. 1. Correlation between urinary 17-ketogenic steroids (ordinate, 17-KGS, mg./24 hrs.) and 17-hydroxycorticosteroids (abscissa, 17-OHCS, mg./24 hrs.). Comparison of 100 simultaneous estimations on different urine specimens.

almost complete (Plantin, to be published). The steroids isolated are the expected end-products of reduction and $NaBiO_3$-treatment of the main urinary 17-OHCS, *i.e.*, tetrahydrocortisone, tetrahydrocortisol, tetrahydro-17-hydroxycortexone (tetrahydro-S), cortol, cortolone, pregnane-3α,17α,20-triol and pregnane-3α,17α,20,21-tetrol. Since the bare isolation of compounds only shows that the extracts contain *some* corticosteroids, but does not indicate how much, attempts were also made to assess the quantitative aspects of specificity. In 15 separate experiments it was found that, on the average, 80 per cent of the estimated 17-OHCS could be attributed to various isolated and identified corticosteroid oxidation products. We consider this as satisfactory evidence of specificity.

Precision. This was assessed on the basis of 688 duplicate estimations of 17-OHCS at different levels of excretion. The standard deviations calculated from these duplicates, using formula I, are compiled in Table 1.

In the range of 10.0–80.0 mg. per day the standard deviation is less than 10 per cent, which is a highly satisfactory precision for urinary steroid assays (25). It is of interest

TABLE 1. ESTIMATES OF PRECISION OF THE METHODS EMPLOYED FOR THE ESTIMATION OF URINARY 17-HYDROXYCORTICOSTEROIDS (17-OHCS) AND 17-KETOSTEROIDS (17-KS), RESPECTIVELY

Precision is expressed in the form of standard deviation (S.D.) of results from their means (from duplicate determinations)

Range (mg./24 hrs.)	17-OHCS		17-KS	
	No. of duplicate determinations	S.D.	No. of duplicate determinations	S.D.
0.0– 2.4			54	0.17
2.5– 4.9			117	0.22
0.0– 4.9	38	0.82		
5.0– 9.9	116	0.76	227	0.42
10.0–19.9	297	1.09	183	0.61
20.0–39.9	156	1.64	46	0.95
40.0–79.9	81	2.08		

that even at pathologically low levels, for instance below 5.0 mg. per twenty-four hours, the S.D. still does not exceed 25 per cent of the estimate.

Accuracy. Since no steroid conjugates were available to us, the accuracy was tested only in recovery experiments—adding 50-μg. amounts of pregnane-3α,17α,20-triol or tetrahydrocortisone to urine samples prior to the reduction step. Following the addition of pregnanetriol, the mean recovery $\pm$S.D. was: 88.2 ± 7.4 per cent (24 experiments), whereas following the addition of tetrahydrocortisone the mean recovery $\pm$S.D. was 88.4 ± 6.0 per cent (5 experiments). These data seem to offer reliable proof that the accuracy of the present method is satisfactory (see, for instance, Marrian (25).

Sensitivity. This was calculated from the S.D. of 38 duplicate estimates at low excretion levels (less than 5.0 mg. per twenty-four hours). It is realized that recovery experiments conducted at low levels probably provide a better assessment of sensitivity than

bare estimates of precision. However, since the standard deviation (S.D.) at this level was 0.82 mg., the 95 per cent fiducial limits can be calculated as ±1.7 mg. Thus it would appear that estimates of urinary 17-OHCS below 2.0 mg. per twenty-four hours or so, probably have very little, if any quantitative significance.

Estimation of urinary 17-ketosteroids

The micromethod of Vestergaard (26), which is based on Callow's absolute alcohol technique (27), was used with slight modifications, including the use of the color correction formula of Allen (22).

Reliability of the method

Specificity. This was tested by infrared spectroscopy following chromatographic separation of the constituents of the ketonic fraction. The main steroids identified were DHEA; A+E; 3α,11β-dihydroxy-etiocholan-17-one; 3α-hydroxy-etiocholane-11,17-dione; and 3α-hydroxy-9-etiocholen-17-one (24). In 37 instances in which chromatographic separation of the 17-KS was performed, it was found that, on the average, 84 per cent of the total Zimmermann chromogenicity could be attributed to identifiable individual 17-KS.

Precision. The standard deviations calculated from 627 duplicates are summarized in Table 1. It is clear from these data that the S.D. is around, or less than 10 per cent.

Accuracy. This was tested in recovery experiments, adding small amounts (30 μg.) of *A+E* to urine after hydrolysis as well as to urine prior to hydrolysis. Thirty experiments in which the steroids were added after hydrolysis resulted in a mean recovery of 83.7±5.8 (S.D.) per cent, whereas in 20 experiments in which the steroids were added prior to hydrolysis, a mean recovery of 80.8±11.6 per cent was obtained. However no recovery experiments were carried out with 11-0-17-KS. It is possible that such experiments would yield less favorable results.

Sensitivity. This was calculated from the S.D. of 54 duplicate estimates at low levels (less than 2.5 mg. per twenty-four hours). Since the S.D. at this level was 0.17 mg., the 95 per cent fiducial limits were calculated as 0.35 mg. per twenty-four hours. Thus estimates of urinary 17-KS below 0.4 mg. per twenty-four hours or so have probably very little quantitative significance.

Separate estimation of dehydroepiandrosterone, androsterone+etiocholanolone and 11-oxygenated 17-ketosteroids

The chromatographic separation of these steroids was performed on the ketonic fraction, following hydrolysis with sulphuric acid, as described in detail previously (24).

Reliability criteria

In these groups of steroids the assessment of reliability criteria was restricted to the chromatographic method of separation.

Specificity. The fractions obtained by this method were pure enough to yield good infrared spectra. It was therefore possible to check the identity of the compounds measured by pooling extracts from different patients. Since any major contamination of these fractions would considerably modify the infrared spectra, the specificity of the method is considered to be high.

Precision. This was calculated from 35 duplicate chromatograms. In each instance

the equivalent of 400 μg. of total 17-KS was chromatographed. The S.D. values were: 0.10 mg. for DHEA, 0.11 mg for A+E, and 0.10 mg. per twenty-four hours for 11-0-17-KS, respectively. This corresponds to 15 per cent of the estimated mean DHEA value, 4 per cent of the mean A+E value, and 13 per cent of the mean 11-0-17-KS value.

Accuracy. This was studied by adding 20-μg. to 140-μg. amounts of the steroids studied to the extracts prior to chromatography. The mean recoveries (±S.D.) were: 99.6±14.3 per cent for DHEA (9 experiments), 104.5±14.3 per cent for A+E (13 experiments), and 85.8±15.1 per cent for 11-0-17 KS (15 experiments).

Sensitivity. The values of the standard deviations seem to indicate that, in these groups of steroids, excretion values of 0.25 mg. per twenty-four hours or so probably do not differ significantly from zero.

Other procedures

Circulating eosinophil leukocytes

These were counted according to the method of Larsson and Svensson (28).

Performance of the intravenous ACTH-test

In order to obtain resting levels in duplicate, urine was collected from each patient on the first and second day. At 9:00 a.m. on the third day, a dose of 25 I.U. of a commercial preparation of ACTH (Acton, Ferring Co., Malmö, Sweden) in 500 to 1000 ml. of physiologic saline or 5.5 per cent glucose solution was administered in a continuous intravenous infusion during eight hours. Urine was collected on this day as well as on the fourth and fifth days of experiment, *i.e.*, on the second and third days following administration of ACTH. However, the steroid analyses on the fourth and fifth days were restricted to the estimation of 17-OHCS and 17-KS excretion. Eosinophil leukocytes were counted before the infusion of ACTH and then every second hour up to eight hours.

As emphasized previously, minute amounts of glucose may interfere with the estimation of urinary 17-OHCS. It is therefore preferable to use physiologic saline for the infusion instead of glucose. Since the tranquilizer Meprobamate is also known to interfere with the estimates (29), it is necessary to be sure that this drug is not being administered during the test period.

Stability of urinary steroids during storage

A prerequisite for carrying out the test in hospitals not equipped with facilities for hormone assays is that no major changes take place in the quantity and nature of urinary steroids during collection and transportation. As far as 17-KS are concerned, this problem has been studied previously (24, 26). Since no such data seem to be available on urinary corticosteroids, at the start of this experiment it was necessary to settle this question. Therefore aliquots of 16 urine samples were stored with toluene at room temperature and at 2° C. Specimens were removed for corticosteroid assays on the first, second, third, fifth, and ninth days of storage.[2] The results of these assays are shown in Figure 2.

Each point in the figure indicates the mean of 16 analyses. Since statistical analysis revealed that the slope of the lines did not differ significantly from zero, it is justifiable to conclude that under these conditions no change in the quantity of urinary cortico-

[2] These estimations were carried out by the previously described method for 17-ketogenic steroid estimation (16).

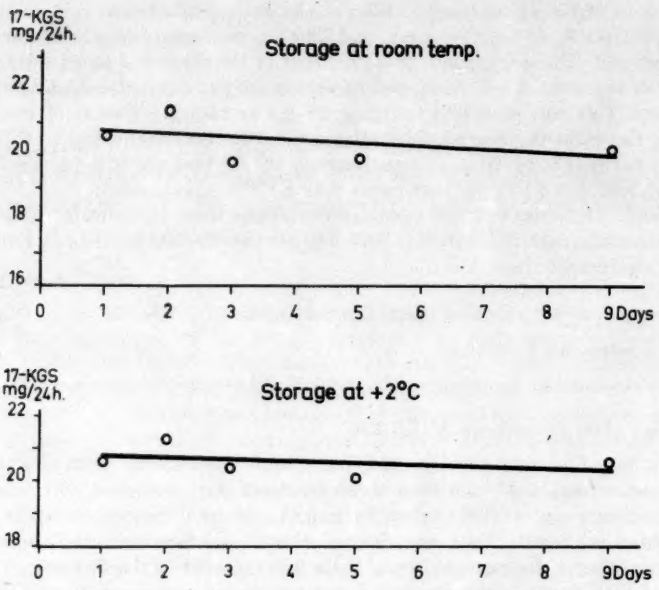

FIG. 2. Influence of storage on the concentration of urinary corticosteroids. Mean values of 16 samples kept under toluene cover at room temperature and at +2° C. (Corticosteroids were estimated as 17-ketogenic steroids (19).)

steroids takes place. Consequently, storage at room temperature up to nine days will not jeopardize the validity of assays.

Day-to-day variation in resting levels of 17-OHCS and 17-KS

From the differences in resting levels between the first and second days of the experiment, standard deviations were calculated, using formula I. The calculations indicated a standard deviation (S.D.) = 2.1 mg. of 17-OHCS per twenty-four hours, which results in a 95 per cent fiducial limit at 4.3 mg. per twenty-four hours. A similar calculation for 17-KS excretion indicated a standard deviation = 1.0 mg. per twenty-four hours, with a fiducial limit at 2.1 mg. per twenty-four hours. Thus a difference between days 1 and 2 exceeding 4.3 mg. of 17-OHCS and 2.1 mg. of 17-KS per twenty-four hours is suggestive of incomplete urine collection.

RESULTS

Excretion of 17-hydroxycorticosteroids

A "typical" ACTH-test. Figure 3 shows the data of a "typical" ACTH-test. The pattern was typical in the sense that it resembled the *average* of all assays in as many respects as possible.

In assessing the effect of ACTH-stimulation, a mean resting level is calculated from the data of days 1 and 2, and this mean value is then contrasted with the "stimulation" value, obtained on the third day. It appears from Figure 3 that in this case the excretion of 17-OHCS increased by 250

per cent following stimulation. It can also be seen that the excretion did not return to a normal value on the fourth day, but did so on the fifth day of the experiment.

Correlation between resting and stimulation levels. In a previous paper (16) it was suggested that in the calculation of resting levels of urinary corti-

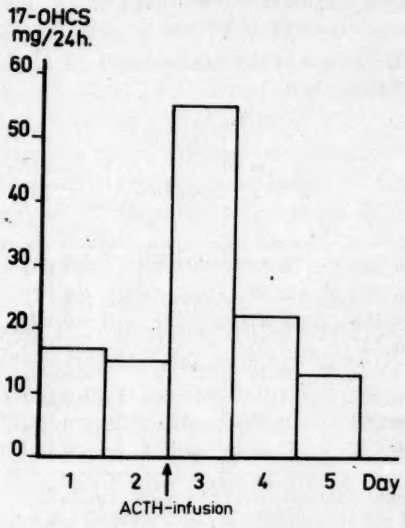

FIG. 3. Results of a "typical" ACTH test.

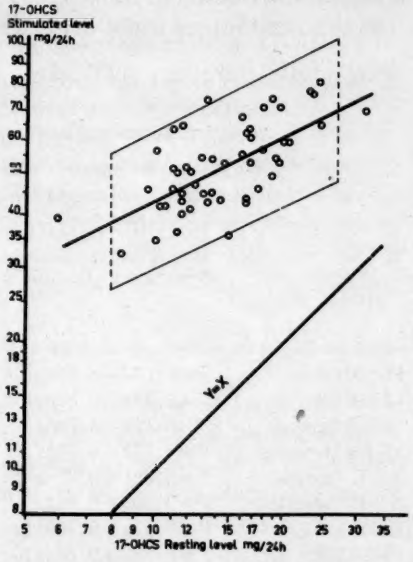

FIG. 4. Relation of resting values to ACTH-stimulation values for urinary 17-hydroxycorticosteroids (17-OHCS) in 52 healthy subjects. The scales are logarithmic, but are graduated in true units. The enframed area corresponds to the fiducial range within which 95 per cent of individual ACTH-test data in healthy subjects are expected to fall.

costeroids, a lognormal (30) rather than normal distribution of individual values should be assumed. The usefulness of this mode of calculation has also been demonstrated by Borth *et al.* (31). In developing the present ACTH test we assumed that not only the resting, but also the stimulation levels are lognormally distributed. Since, in the present material—in contrast to our previous findings—no significant sex difference in 17-OHCS excretion was found, the data obtained in male and female subjects were analyzed together. When the logarithms of mean resting levels were plotted against the logarithms of stimulation levels, the data shown in Figure 4 were obtained.

The equation of the line relating log-resting to log-stimulation excretion values was asserted to be $Y=1.136+0.500x$, as shown in Table 2, where the relevant statistical data from the analysis of all steroids involved are summarized.

It also follows from the data of Table 2, that the regression coefficient is significantly different from zero ($P<0.001$). Control calculation revealed that this significance is not influenced by the sex of subjects. Hence, since

TABLE 2. SUMMARY OF THE EQUATIONS OF LINES RELATING THE LOGARITHMS OF ACTH-STIMULATION VALUES TO THE LOGARITHMS OF RESTING VALUES OF DIFFERENT URINARY STEROIDS OR STEROID GROUPS

Some statistics required for the assessment of the significance of regression are also shown.

Steroid, or steroid group	Equation of the line	s_b*	b/s_b**	Degrees of freedom (D.F.)	Probability (P)
17-OHCS	$Y=1.136+0.500x$	0.083	6.02	50	<0.001
17-OHCS†	$Y=0.693+0.513x$	0.152	3.38	50	<0.01
17-KS (males)	$Y=0.472+0.785x$	0.108	7.27	24	<0.001
17-KS (females)	$Y=0.571+0.705x$	0.131	5.38	24	<0.001
A+E (males)	$Y=0.621+0.775x$	0.127	6.10	16	<0.001
A+E (females)	$Y=0.665+0.782x$	0.132	5.92	18	<0.001
DHEA	$Y=0.438+0.993x$‡	0.079	12.57	33	<0.001
11-0-17-KS	$Y=0.753+0.555x$‡	0.132	4.20	34	<0.001

* Standard error of the regression coefficient. Calculated as shown in formula II.

** Ratio between the regression coefficient and its standard error. This ratio provides an estimate of *t*.

† Excretion values on the day following ACTH-stimulation (fourth day).

‡ For the sake of convenience in the computational work, all logarithms have been increased by 1.0. The equations refer to such increased values, whereas Figures 6 to 8 show the real values.

the regression of the log-stimulation 17-OHCS value upon the log-resting value is highly significant, it may be concluded that the excretion value of 17-OHCS following intravenous ACTH-stimulation is a function of the resting level. Under such circumstances it appears to be necessary to calculate approximate fiducial limits around the regression line (formula IV). These limits are also indicated in Figure 4. An examination of these limits reveals that there is a fairly wide area between no response ($x=y$) and the lower fiducial limit of the normal response. It is concluded, therefore, that the present design also provides conditions for the laboratory diagnosis of latent adrenocortical hypofunction.

When the resting levels were plotted in a similar fashion against the values obtained on the day following ACTH-infusion (day 4), there was still a significant regression (Table 2). Although the 17-OHCS values were greatly reduced on the fourth day when compared with those obtained on the third day, they still were elevated when compared to the resting levels. This is interpreted as an indication that the applied dose of ACTH was in excess of that required for maximal stimulation.

It appeared also of interest to calculate the mean ratio of 17-OHCS excretion between the third and fourth days. This was found to be 2.78, indicating that the average subject will excrete 2.8 times more 17-OHCS on the day of ACTH-infusion than on the day after infusion. Using formula II, the standard deviation of this ratio was calculated as 0.856, which with a 0.05 probability deviate (45 d.f.), provides fiducial limits to the above statement at 1.05 and 4.51, respectively. Thus a ratio of 1.0, or less, appears to be suggestive evidence of an abnormal result with the ACTH test. The clinical significance of this ratio will be discussed in another paper (Birke *et al.*, to be published).

Excretion of 17-ketosteroids

Inspection of the data of Hamburger (32) and those of Lugg and Bowness (33) suggested to us that urinary 17-KS also may be lognormally rather than normally distributed. Therefore a design similar to that shown in Figure 4 was used in this case, with the exception that—due to the highly significant sex difference in resting levels—separate lines were calculated for males and females. The regression lines and fiducial limits are shown in Figure 5, a and b.

It appears from the data in this figure that there is virtually no space between lack of response ($x=y$) and the lower fiducial limit of normal response. In a considerable number of cases the increase in urinary 17-KS following ACTH-stimulation was very slight. Since the same subjects exhibited a marked response to ACTH as far as the excretion of 17-OHCS is concerned, it may be concluded that the excretion of 17-KS does not provide a sufficiently sensitive test for the assessment of adrenocortical reserve. This is in accord with the view expressed by earlier investigators (*e.g.*, (2)). It is of interest, however, that the stimulation 17-KS values, both in males and females, showed the same highly significant correlation to resting levels, as the 17-OHCS values (Table 2). It should also be noted that the regression coefficient for males did not differ significantly from that for females. This is not surprising, since testicular steroid production is not expected to be influenced by ACTH. Finally, it is of interest that the 17-ketosteroids had returned to resting levels on the fourth day of the experiment.

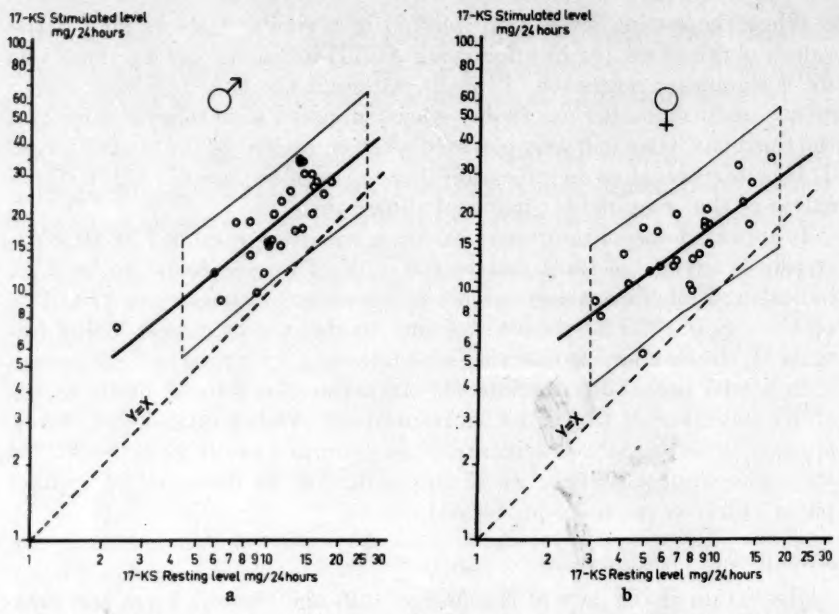

FIG. 5. Relation of resting values to ACTH-stimulation values for urinary total 17-ketosteroids (17-KS)— **a**) in healthy males, and **b**) healthy females. For further particulars, consult Figure 4.

Excretion of androsterone+etiocholanolone

Since also in this case a significant sex difference was found, the data obtained in males and females, respectively, were treated separately. The results are shown in Figure 6, a and b.

An examination of the statistical data of Table 2 reveals that here also there is a significant correlation between resting levels and stimulation levels. It appears from the data of Table 2 that the value of the regression coefficient in males and females, respectively, is almost identical. This indicates that following ACTH-stimulation the increased output of A+E in males is most probably entirely of adrenal origin. Examination of the fiducial limits shown in Figure 6 also reveals that the estimation of A+E, in itself, is not a useful test of adrenocortical reserve. It may, however, be a very useful test in the diagnosis of certain forms of adrenocortical hyperfunction.

Excretion of dehydroepiandrosterone

In this case no significant sex difference was found. The combined data for males and females are summarized in Figure 7.

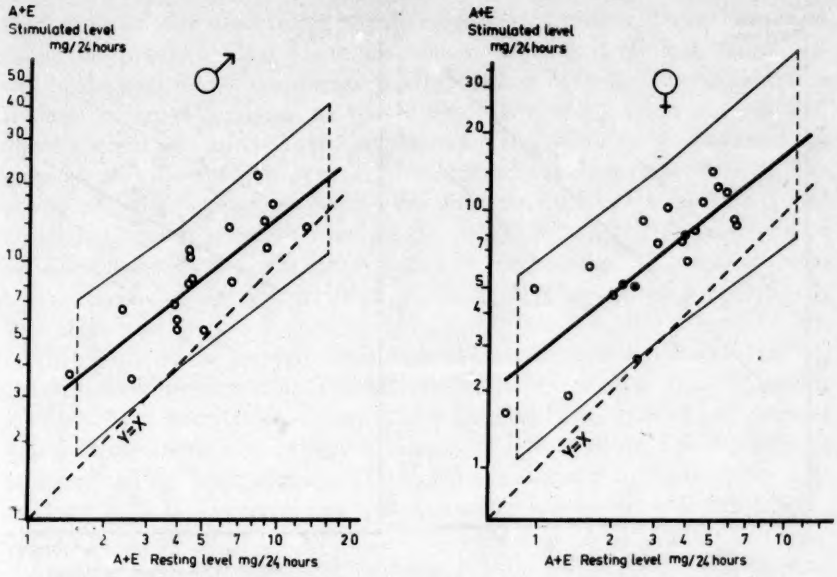

FIG. 6. Relation of resting values to ACTH-stimulation values for urinary androsterone+etiocholanolone (A+E)—**a**) in healthy males, and **b**) healthy females. For further particulars, consult Figure 4.

Also in this instance the correlation between resting and stimulation levels was very high (Table 2). It is of interest that, among all steroid groups studied, the slope of the regression line was steepest in this test. It is apparent that there was a very wide variation in resting levels, and that there was an overlapping between no response and the lower limit of normal response—indicating that estimation of DHEA as a general test of adrenocortical reserve is of little value. However, the establishment of the upper limit is of importance as far as further studies on hyperfunction are concerned.

Excretion of 11-oxygenated-17-ketosteroids

No difference between sexes was found as far as the excretion of this group of steroids is concerned. The combined data are shown in Figure 8.

Statistical analysis reveals that the correlation between resting and stimulation levels was highly significant in this case also (Table 2). However, examination of the data of Figure 8 shows that estimation of this particular group of urinary steroids does not provide a useful test for the assessment of adrenocortical reserve.

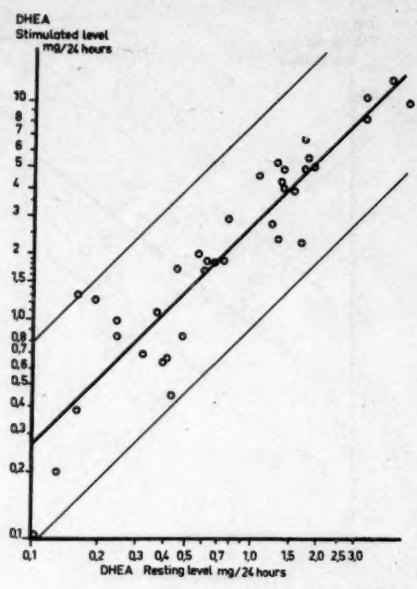

FIG. 7. Relation of resting values to ACTH-stimulation values for urinary dehydroepiandrosterone (DHEA) in healthy subjects. For further particulars, consult Figure 4.

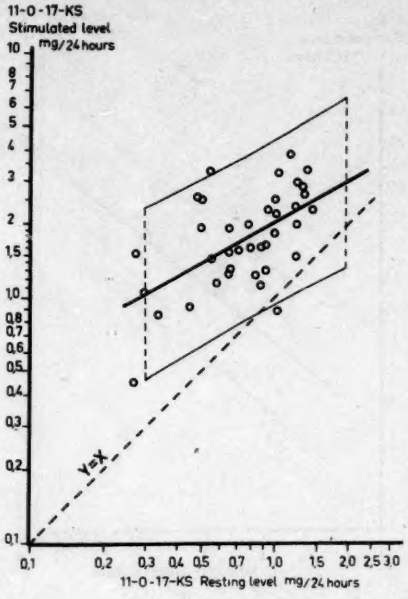

FIG. 8. Relation of resting values to ACTH-stimulation values for urinary 11-oxygenated-17-ketosteroids (11-0-17-KS) in healthy subjects. For further particulars, consult Figure 4.

Eosinopenic reaction

This was studied in 44 subjects, but in 9 of them the resting eosinophil level was below 100 cells per cu. mm. These 9 were not included in the present study due to the enormous error involved in the calculation of the percentage reduction in such cases. In the remaining 35 cases the mean percentage reduction ($\pm$S.D.) was 87.7 ± 20.2, which agrees well with the data reported by Jenkins *et al.* (2). In 3, the reduction was less than 50 per cent, although the estimation of 17-OHCS excretion indicated a satisfactory response to ACTH. Thus in 12 out of 44 apparently normal subjects exhibiting normal steroid response (*i.e.*, in approximately one third of the cases) the eosinopenic reaction proved to be useless or misleading as a criterion of the adrenocortical response to ACTH.

DISCUSSION

In assessing the overall value of a clinical laboratory method for determining adrenocortical reserve, it seems to be of paramount importance

that the methods used fulfill certain essential requirements, such as reliability and practicability. There has been a tendency in clinical endocrinology in the past to overemphasize the importance of factors such as simplicity and speed of analysis, at the expense of reliability. In our opinion, equally great emphasis should be placed on the reliability of the methods employed and on the proper establishment of "normal material." The purpose of the latter is to enable the endocrinologist to make predictive fiducial statements, whether or not the results of a particular test can be considered as normal. The approximative character of all kinds of "normal material" and of fiducial limits needs perhaps some special emphasis in this respect.

The results of the present study indicate a high degree of correlation between resting levels and ACTH-stimulation levels for all groups of steroids studied. This correlation excludes the possibility of calculating fiducial limits of the increment in steroid excretion following ACTH-stimulation as suggested by Jenkins *et al.* (2), since if the stimulation value (y) be correlated to the resting level (x), then the difference ($y-x$) will still be correlated to x.

Another possibility would be to express the steroidogenic response to ACTH as the ratio of urinary 17-OHCS output after ACTH-stimulation to "basal" (resting) output. Such a ratio has been used by DiRaimondo *et al.* (34) as an effect metameter in the bioassay of ACTH in human subjects. However, in the present study this ratio also exhibits a highly significant correlation to resting levels. (The equation of the regression line is: $Y=5.39-0.112x$, where x denotes the resting level and y is the ratio.) Our approach to the problem involves the assumption that the logarithms of resting and stimulation levels are normally distributed. We feel that this is a relatively minor and most probably justifiable assumption. It is of interest, however, that even when the mode of calculation suggested by Jenkins *et al.* (2) is applied to the present data, there is no overlapping between lack of response and the lower limit of normal response. In interpreting this finding it should be remembered that the present technique estimates 4 types of corticosteroid side-chains.

By the use of previously published results of ACTH-tests, it has been possible to differentiate adrenocortical hyperfunction from normal states, but the laboratory diagnosis of adrenocortical hypofunction sometimes has been difficult. Using the present technique it is possible to delineate an area also for the diagnosis of subnormal response to ACTH in cases in which the resting level is apparently normal. The significance of this factor in the evaluation of abnormal response will be discussed in another paper (Birke *et al.*, to be published).

A comparison of the data obtained by the different methods used in this study reveals that by far the best indicator of adrenocortical functional capacity is the estimation of 17-OHCS excretion following ACTH-stimulation. Our findings confirm the conclusions of previous investigators (2) that urinary 17-KS excretion is a poor and unreliable index of the functional capacity of the adrenal cortex. Although estimations of this and other steroid groups will thus in no way replace the 17-OHCS test, their use might offer valuable additional information in cases of adrenocortical dysfunction, such as virilism or hirsutism. The usefulness of the eosinopenic response as an indicator of adrenocortical functional reserve seems to be very limited. The relatively low specificity of this method has already been pointed out by previous investigators. In the present study this reaction failed to yield the expected information in approximately 30 per cent of apparently normal cases. Under such circumstances it is hardly justifiable to place too much reliance on this method, except in emergency situations.

It is realized, that the ACTH-test suggested in this paper is time-consuming. However, provided the completeness of urine collection is under careful control, we recommend a simplified test involving a control period of only one day, and a study period of two days during and after infusion.

It is also realized that the intramuscular administration of long-acting depot preparations of ACTH instead of intravenous infusion would be of great advantage. Such preparations have been employed by other investigators (2, 34, 35). It is likely that in the not too distant future such long-acting preparations will replace the intravenous method. However, for the time being, the differences in the action of various types of preparations, the existing difficulties in their routine standardization, and the lack of suitable normal values are serious arguments against the use of long-acting ACTH preparations for other than therapeutic purposes.

Acknowledgment

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ABSORPTION OF ACETATES OF HYDROCORTISONE, Δ^1-HYDROCORTISONE AND CORTISONE FROM THE JOINT CAVITY INTO THE CIRCULATION

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ABSTRACT

Absorption of the acetates of hydrocortisone, Δ^1-hydrocortisone and cortisone from the synovial cavity of the knee into the circulation was studied in 28 patients suffering from rheumatoid arthritis. A comparison was also made of the oral and intraarticular absorption of hydrocortisone acetate, by administering equal doses by both routes to a group of patients. The acetates of hydrocortisone, Δ^1-hydrocortisone and cortisone were found to be absorbed from the joint cavity into the circulation in significant quantities. The maximal free plasma 17-hydroxycorticosteroid levels were reached within three to six hours after the injection. At twenty-four hours there were still detectable amounts of injected steroids in the circulation in most of the cases. No major differences could be observed in the plasma concentrations of free 17-hydroxycorticosteroids after intraarticular administration of equal doses of the 3 steroids. Individual variations in each group were, however, pronounced. The elevation of conjugated plasma 17-hydroxycorticosteroids after intra-articular injection of cortisone acetate was much greater than after the injection of hydrocortisone acetate. No significant elevation in conjugated plasma 17-hydroxycorticosteroids could be demonstrated after intra-articular injection of Δ^1-hydrocortisone acetate. The absorption of hydrocortisone acetate into the blood after oral administration was much greater and more rapid than after intra-articular injection. The disappearance of orally administered hydrocortisone acetate from the plasma was also more rapid than that of the intra-articularly injected steroid.

IT HAS been reported in an earlier paper by the author (1) that hydrocortisone acetate (F-ac.) is absorbed from the joint cavity into the circulation to a considerable degree. This gave rise to the question as to whether any differences could be shown in the absorption of different corticosteroids from the joint cavity, which might explain the dissimilar intra-articular effectiveness of these hormones. It is well known that intra-articularly injected cortisone acetate (E-ac.) has very little local antirheumatic potency (2–4). On the other hand, Δ^1-hydrocortisone acetate (Δ^1-F-ac.) has a much greater intra-articular effect than F-ac.

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The rise in the level of plasma 17-hydroxycorticosteroids (17-OHCS) after intra-articular use of corticosteroids also has clinical significance, because systemic hormonal effects are recorded in connection with local F-ac. therapy (5, 6).

The aim of the present investigation was to clarify these problems by studying the effect of intra-articular injections of F-ac., Δ-F-ac. and E-ac. on the plasma levels of free and conjugated 17-OHCS.

MATERIAL AND METHODS

Twenty-eight patients with rheumatoid arthritis were studied. The corticosteroids were injected into the knee joints. Endogenous hydrocortisone production was suppressed by administering Δ^1-9-α-fluorohydrocortisone in a dosage of 0.5 mg. orally every six hours for two days before the intra-articular injections and thereafter until the last blood specimen was taken (7). The first blood sample was withdrawn immediately before the intra-articular injection at 8 a.m. Subsequent samples were drawn at varying intervals up to twenty-four hours.

A comparison was also made between oral and intra-articular absorption of F-ac. by administering to a group of patients equal doses by both routes.

The plasma levels of free 17-OHCS were determined by Silber and Porter's method (8) as modified by Peterson *et al.* (9, 10). To estimate the level of conjugated plasma 17-OHCS, the following procedure was used: 400 units of bacterial β-glucuronidase (Sigma) in 1 cc. of redistilled water, 0.5 cc. of 1 *M* K_2HPO_4 (pH 4.15) and 0.3 cc. of 5 per cent $NaHSO_3$ were added to 5 cc. of plasma and incubated at 37° C. for forty-eight hours. Sodium bisulphite was added according to the recommendation of Louis *et al.* (11), to inhibit the formation of interfering material during enzymatic hydrolysis in the presence of glucose. After incubation the method was the same as for free plasma 17-OHCS. In this way the level of total plasma 17-OHCS was measured. To obtain the level of conjugated plasma 17-OHCS, the value for free 17-OHCS was subtracted from the value for total 17-OHCS.[1]

The results are expressed as increases in plasma 17-OHCS concentration (μg./100 ml.) from the depressed control value. The level of free plasma 17-OHCS after two days of Δ^1-9-α-fluorohydrocortisone administration ranged from 0 to 10 μg./100 ml. (mean, 3.2 μg./100 ml.). The corresponding figures for conjugated plasma 17-OHCS ranged from 0 to 6 μg./100 ml. (mean, 1.5 μg./100 ml.).

RESULTS

Absorption of hydrocortisone acetate

After intra-articular injection of 50 mg. of F-ac. in 13 patients with rheumatoid arthritis, there was a clear rise in the level of free plasma 17-OHCS in all but 1. The maximal levels were reached after three to six hours. A decrease in plasma 17-OHCS concentration occurred after twelve to twenty-four hours. In most instances, part of the intra-articularly injected steroid was still circulating in the blood twenty-four hours after injection.

[1] A detailed report of the method for determination of conjugated plasma 17-OHCS will be published in *Scandinav. J. Clin. & Lab. Invest.* **10:** (Oct.) 1958, by F. E. Krusius and M. Oka.

Considerable individual variation in the rate of absorption was noted.

After intra-articular injection of 100 mg. of F-ac. in 7 patients, there was a marked elevation of free plasma 17-OHCS concentration in all instances. The mean rise in concentration after three hours differed significantly from the corresponding mean for the group in which the dose was 50 mg. After six to twenty-four hours the differences were still statistically slightly significant (Fig. 1).

The rise in the level of plasma conjugated 17-OHCS was measured in 4 cases after intra-articular injection of 50 mg. of F-ac. and in 4 cases after injection of 100 mg. of F-ac. The rate of conjugation seemed to be slow. The highest levels were reached in most instances after twenty-four hours (Fig. 2).

A comparison between oral and intra-articular absorption of F-ac. was made in 6 instances. Only the levels of free 17-OHCS were determined. It

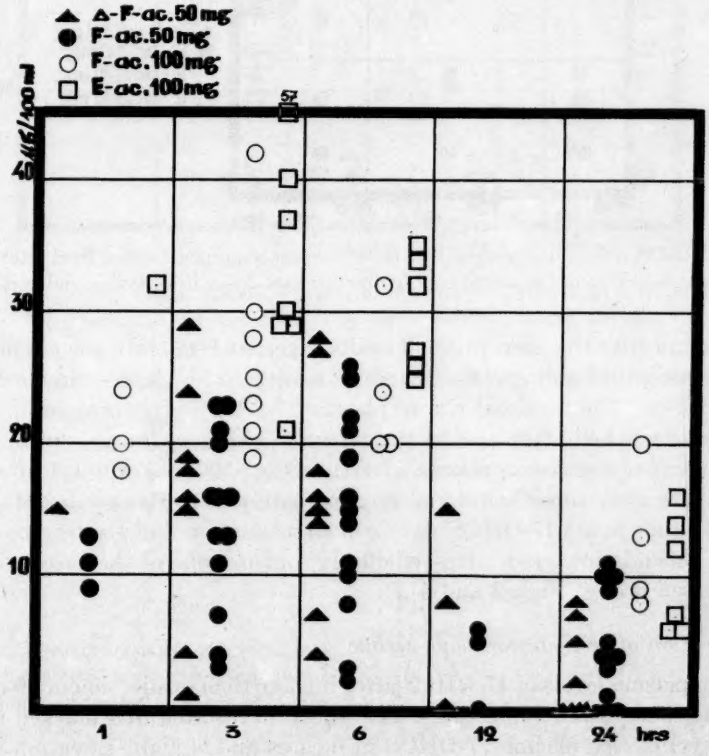

FIG. 1. Rise in free plasma 17-OHCS from the suppressed initial level after intra-articular injection of the acetates of hydrocortisone, Δ^1-hydrocortisone and cortisone.

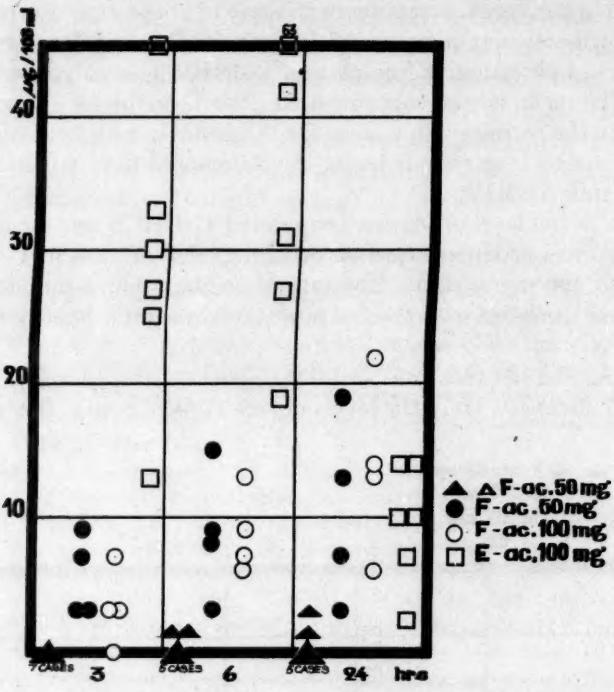

FIG. 2. Rise in conjugated plasma 17-OHCS from the suppressed initial level after intra-articular injection of the acetates of hydrocortisone, Δ^1-hydrocortisone and cortisone.

was found that the absorption of orally ingested F-ac. into the circulation was more rapid and greater than the absorption of intra-articularly injected F-ac. The maximal rise in plasma 17-OHCS after oral administration was reached after one to three hours. The mean ratio, "oral/intra-articular," of the rise in plasma 17-OHCS (μg./100 ml.) was 3:1 after one hour, 2:1 after three hours and approximately 1:1 after six hours. After twenty-four hours 17-OHCS were still circulating in 5 of the 6 cases when F-ac. was administered intra-articularly, but in none of the cases when it was given orally (Figs. 3 and 4).

Absorption of Δ^1-hydrocortisone acetate

The plasma levels of 17-OHCS after intra-articular injection of 50 mg. of Δ-F-ac. were studied in 8 cases. The injection resulted in a marked rise in the level of free plasma 17-OHCS in 6 cases and a slight elevation in the remaining 2. The maximal levels were attained three or six hours after intra-articular injection. The mean rise in the level of the free plasma

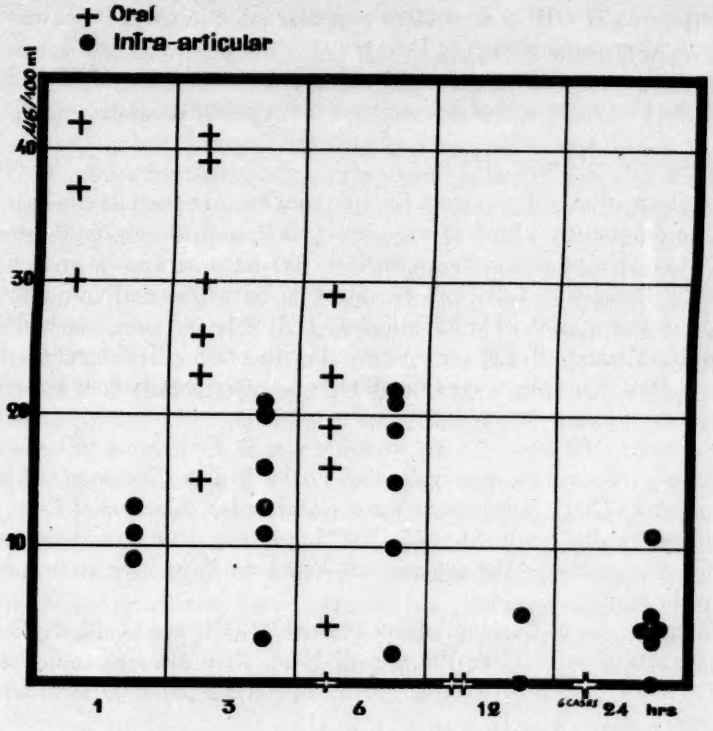

FIG. 3. Rise in free plasma 17-OHCS from the suppressed initial level after oral and intra-articular administration of 50 mg. of hydrocortisone acetate.

corticosteroid fraction was of the same magnitude as after the injection of 50 mg. of F-ac. (Fig. 1).

In 2 of the cases the absorption of 50 mg. of F-ac. from the joint cavity into the circulation was also studied. In the first case the absorption of the 2 steroids was about the same, but in the second the rise in concentration

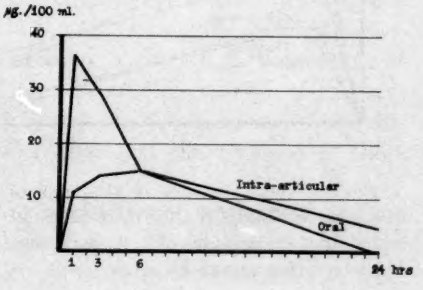

FIG. 4. Mean changes in the level of free plasma 17-OHCS after oral and intra-articular administration of 50 mg. of hydrocortisone acetate.

of free plasma 17-OHCS after intra-articular injection of Δ-F-ac. was much less than after the injection of F-ac.

No significant rise in the level of conjugated plasma 17-OHCS after intra-articular injection of Δ-F-ac. was observed (Fig. 2).

Absorption of cortisone acetate

The absorption of E-ac. from the joint cavity into the blood was investigated in 8 patients. The dose was 50 mg. in 2, and 100 mg. in 6 instances. The data on the patients who were given 100 mg. each are shown in Figures 1 and 2. There was a distinct elevation in both free and conjugated 17-OHCS of the plasma. The maximal 17-OHCS levels were reached within three to six hours after the intra-articular injection. The concentrations of both 17-OHCS fractions were much reduced after twenty-four hours (Figs. 1 and 2).

In 3 cases, 100 mg. of E-ac. and 100 mg. of F-ac. were injected intra-articularly on separate occasions. In 2 of the 3 cases the rise in the level of free plasma 17-OHCS was clearly greater after the injection of E-ac. When the effects on the conjugated 17-OHCS fractions were compared, the difference was marked—the conjugation being much greater after the injection of E-ac.

The mean rise in the level of free plasma 17-OHCS was slightly, but not significantly greater after 100 mg. of E-ac. than after a similar dose of F-ac. The difference between the levels of conjugated 17-OHCS, however, was marked.

The mean changes in the concentration of free and conjugated 17-OHCS in the plasma after intra-articular injection of hydrocortisone acetate, Δ^1-hydrocortisone acetate, and cortisone acetate are shown in Figures 5 and 6.

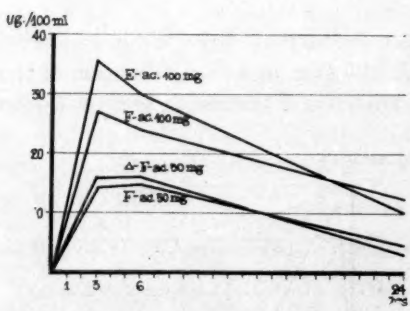

FIG. 5. Mean changes in the level of free plasma 17-OHCS after intra-articular injection of the acetates of hydrocortisone, Δ^1-hydrocortisone and cortisone.

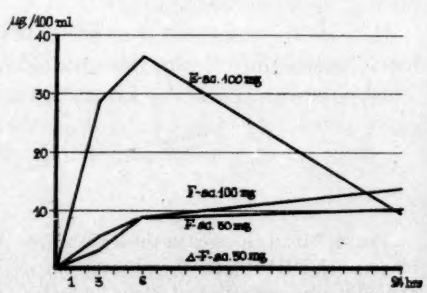

FIG. 6. Mean changes in the level of conjugated plasma 17-OHCS after intra-articular injection of the acetates of hydrocortisone, Δ^1-hydrocortisone and cortisone.

DISCUSSION

It has been shown previously that intra-articularly injected hydrocortisone, cortisone and their acetates disappear rapidly from the joint cavity (12–15). According to Wilson *et al.* (13), about 85 and 98 per cent of the injected steroids had disappeared after one hour and three hours, respectively. Metabolite formation within the synovial cavity was also observed.

In the light of the present investigation, it seems that this rapid disappearance is caused to a great extent by absorption from the synovial cavity into the circulation. All of the steroids studied appeared in the plasma in significant quantities three to six hours after the intra-articular injection. The rise in plasma 17-OHCS concentration was influenced by the size of the dose used. When a 100-mg. dose of F-ac. or E-ac. was injected intra-articularly, the elevations of plasma 17-OHCS were of the same magnitude as can be obtained after maximal stimulation of the adrenal cortex by intravenous infusion of ACTH (16–19).

Bearing this fact in mind, it is easy to understand how systemic hormonal effects have been observed after intra-articular use of F-ac. These have manifested themselves by improvement in involved joints not treated by injection of steroid (5); and also by changes in sugar metabolism (6).

A distinct difference was found between oral and intra-articular absorption of F-ac. The absorption of intra-articularly injected F-ac. was markedly delayed compared with the absorption after oral administration. It seems therefore likely that a part of the steroid is accumulated in the synovial tissue, where it acts locally. The other part easily passes through the joint fluid-blood barrier into the circulation, where it has general systemic effects.

The absorptions of F-ac. and Δ F-ac. from the joint cavity into the blood did not seem to differ much from one another. The actual levels of free plasma 17-OHCS after intra-articular injection of Δ-F-ac. were, however, somewhat higher than the figures indicate, because the chromogenicity of Δ F-ac. is two-thirds that of F-ac. in the Porter-Silber reaction (20).

The intra-articular injection of E-ac. caused a slightly greater rise in the level of free plasma 17-OHCS than did the injection of F-ac., and the rise in the level of conjugated plasma 17-OHCS was much greater. This is evidence of the faster rate of metabolism of E-ac. A similar phenomenon was previously demonstrated by means of studies on the half-life of intravenously injected hydrocortisone and cortisone (9, 21). This metabolic difference may also have some influence on the local effectiveness of these steroids.

The failure to demonstrate significant quantities of conjugated plasma 17-OHCS after intra-articular injection of Δ^1-F-ac. shows that the Δ^1-F-ac. which is absorbed into the blood mainly remains unmetabolized and free.

This is in accordance with the report of Slaunwhite *et al.* (20), who found that the Δ^1-17-OHCS are excreted in the urine largely unconjugated and for the most part unchanged. They suggested also that the greater clinical effectiveness of Δ^1-17-OHCS may be related to the slower metabolism and inactivation of these compounds.

Acknowledgment

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DIURNAL VARIATION OF PLASMA 17-HYDROXY-CORTICOSTEROIDS IN SUBJECTS SUFFERING FROM SEVERE BRAIN DAMAGE*

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ABSTRACT

Discrepancy from the normal diurnal variation of plasma 17-OHCS has been observed in 4 human subjects suffering from various forms of severe damage to brain tissue associated with a profound degree of impairment of consciousness. A number of explanatory hypotheses as to the mechanism for diurnal variation of plasma 17-OHCS have been reviewed. Although no definite conclusions can be drawn from the data presented, they suggest that higher central nervous system processes *may* play a role in the regulation of 17-OHCS.

THE occurrence of a diurnal rhythm in urine and plasma levels of 17-hydroxycorticosteroids (17-OHCS) in man has been previously demonstrated by several investigators (1–3). Further studies have indicated that in subjects who had worked irregularly on night shifts for six months or longer and in totally blind subjects there were no significant changes in this diurnal rhythm (4). No satisfactory explanation of the mechanism or evaluation of the significance of diurnal variation is available.

The present investigation was carried out on the assumption that processes in the central nervous system involved in awareness of rhythmic changes in the external environment might be a necessary condition for the normal diurnal variation in plasma 17-OHCS levels. This hypothesis was tested in 4 human subjects with severe central nervous system disorders who had in common a profound degree of impairment of consciousness.

METHODS AND SUBJECTS

Blood samples were withdrawn into heparinized tubes and centrifuged immediately. The plasma was drawn off and deep-frozen. Plasma 17-OHCS were determined as described by Eik-Nes (5).

The investigation was carried out on 4 patients with serious brain damage (Table 1). Two normal persons, aged 35 and 49, served as controls. They were kept at complete rest in bed during each 24-hour study period and were given the same diet (a tube-feeding

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TABLE 1. CLINICAL DATA ON 4 SUBJECTS WITH SEVERE NEUROLOGIC ILLNESS

Subject Age	Diagnosis	Electroencephalogram	Ventriculogram or pneumoencephalogram	Neurologic examination	Duration of disturbance of consciousness
A.W., 31	Encephalopathy secondary to trauma (auto accident), with generalized cerebral and cerebellar atrophy	Abnormal: generalized, irregular slow-wave activity	Marked dilation of entire ventricular system including aqueduct of Sylvius and 4th ventricle	No voluntary responses. Spastic quadriplegia (decerebrate rigidity). Bilateral optic atrophy; no pupillary light response. Reflex chewing and swallowing. Incontinent. No definite sleep-wakefulness pattern. Bedfast and tube-fed.	3 yrs.; no improvement
K.C., 32	Chronic meningo-encephalitis—cause undetermined	Abnormal: generalized slowing	Minimal symmetrical cerebral atrophy	No voluntary responses. Spastic quadriplegia (decerebrate rigidity). Painful stimuli produce grimacing and diffuse motor responses. No definite sleep-wakefulness pattern. Incontinent. Bedfast and tube-fed.	2 yrs.; no improvement
J.A., 63	Progressive, bilateral cerebral thrombosis with quadriplegia and pseudobulbar palsy	Abnormal: generalized slowing	Not obtained	Bilateral hemiplegia with aphasia, dysphagia and oculomotor and facial paresis. Generalized spasticity and hyper-reflexia. Totally unable to communicate. Appears to "wake" and "sleep" but in no constant pattern. Incontinent. Bedfast and tube-fed.	2 yrs.; no improvement
R.W., 26	Encephalopathy secondary to trauma (auto accident)	Abnormal: generalized slowing	Moderate cerebral atrophy; left hemisphere more atrophic than right	Quadriplegia, most marked on right. Semi-comatose. Responds to painful stimuli with grimacing and meaningless shouting and screaming. No other meaningful responses. Appears to "sleep" and "wake" but in no constant pattern. Bedfast and spoon-fed.	6 mos.; slight improvement

of a food mixture every six hours) as the patients with brain damage. At least two weeks elapsed between successive diurnal tests on any of the subjects described.

RESULTS

The diurnal variation of plasma 17-OHCS on 3 different occasions in the subjects suffering from cerebral damage is shown in Figure 1. In most cases, these curves differed from the composite diurnal curve based on mean values from a large number of normal subjects (Fig. 2) (4). The mean plasma levels of 17-OHCS were higher in the pathologic cases than in the 2 normal subjects (Table 2), and the pattern in any single pathologic subject varied considerably when repeated at intervals. A fairly reproducible diurnal variation of 17-OHCS was found in the control subjects (Fig. 3).

DISCUSSION

From the data, it appears that serious disorders of the central nervous system associated with loss of awareness of the external environment *may* produce high plasma levels of 17-OHCS and an impairment in the normal diurnal pattern of these steroids. In the patients studied, changes in plasma 17-OHCS *did* occur, but they were erratic—often in no consistent relationship to night or day, and of varied pattern on successive occasions. A nor-

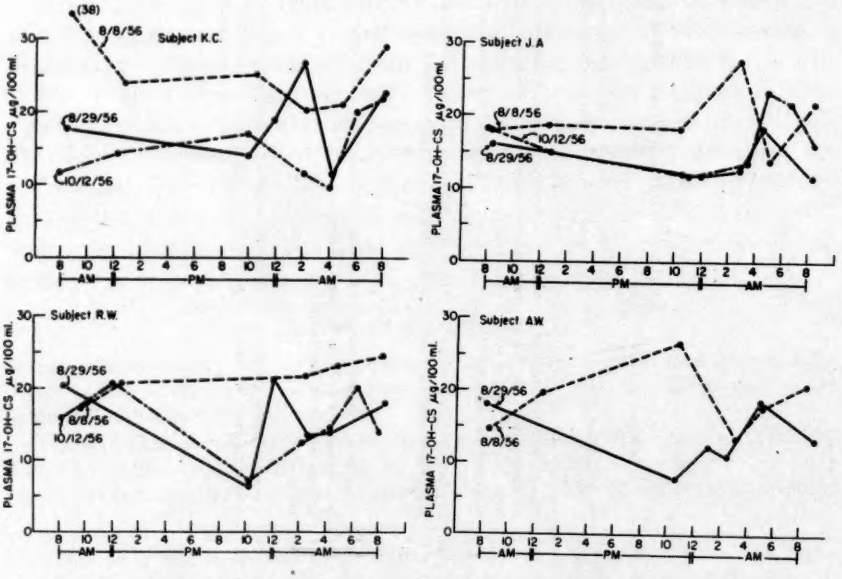

FIG. 1. Diurnal variations of plasma 17-OHCS in 4 patients with severe impairment of consciousness.

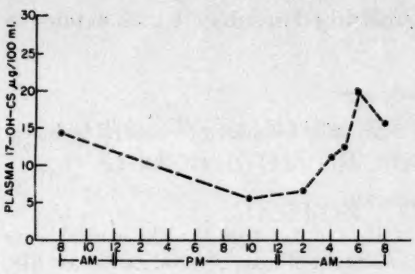

FIG. 2. Mean diurnal variation of plasma 17-OHCS in a large group of normal subjects (4).

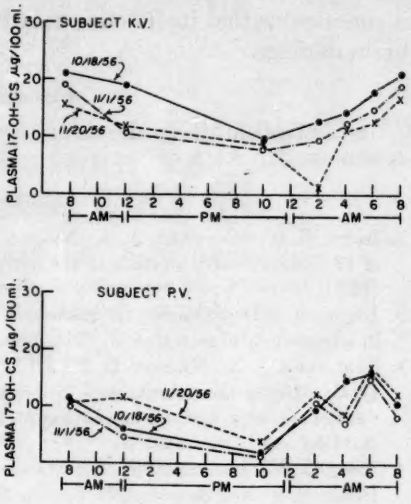

FIG. 3. Plasma levels of 17-OHCS in normal subjects under conditions of bed rest and standardized food intake.

mal diurnal variation was seen only infrequently. This contrasts with a fair reproducibility of the diurnal pattern of normal subjects when studied under comparable conditions (Fig. 3; Table 2).

Some reflection of the diurnal cycle through the central nervous system would therefore seem to be involved. Rauschkolb and Farrell (6) have recently postulated a diencephalic regulation for aldosterone secretion. However, a higher central nervous system mechanism involved in secretion of 17-OHCS has not yet been described.[1] If such a regulatory center exists, it

TABLE 2. STATISTICAL EVALUATION OF MEAN PLASMA LEVELS OF 17-OHCS IN 2 NORMAL SUBJECTS AND 4 PATIENTS WITH SERIOUS BRAIN DAMAGE

Groups compared	Probability	Groups compared	Probability
8 A.M. (s)*–8 A.M. (n)*	>0.05 <0.02	8 A.M. (s)–12 Noon (s)	<0.50
12 Noon (s)–12 Noon (n)	>0.02 <0.01	12 Noon (s)–10 P.M. (s)	>0.10 <0.05
10 P.M. (s)–10 P.M. (n)	>0.02 <0.01	10 P.M. (s)–2 A.M. (s)	>0.50 <0.10
2 A.M. (s)–2 A.M. (n)	>0.02 <0.01	2 A.M. (s)–8 A.M. (s)	>0.50 <0.10
4 A.M. (s)–4 A.M. (n)	>0.05 <0.02	8 A.M. (n)–12 Noon (n)	>0.50 <0.10
		12 Noon (n)–10 P.M. (n)	>0.05 <0.02
		10 P.M. (n)–2 A.M. (n)	>0.05 <0.02
		2 A.M. (n)–8 A.M. (n)	>0.10 <0.05

* (s) = the sick group.
(n) = the normal group.

[1] Recently Mason *et al.* have observed that a disruption in the diurnal variation of urinary 17-OHCS will occur in monkeys subjected to bilateral section of the fornix (*Cf.* Program of the 39th Meeting of The Endocrine Society, Page 61. 1957).

is conceivable that its function may be disturbed in subjects with extensive brain damage.

Acknowledgment

The authors are greatly indebted to Mrs. Kari Brinck-Johnsen for skillful technical assistance.

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PROBENECID IN HYPOPARATHYROIDISM: ABSENCE OF PHOSPHATURIC RESPONSE

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ABSTRACT

The administration of probenecid to each of 4 hypoparathyroid patients during the measurement of specific renal functions did not alter the renal excretion of phosphorus, the clearance of phosphorus, or the concentration of phosphorus in the serum. In contrast, the administration of *p*-aminohippurate to 3 of these subjects was followed by an increased renal excretion of phosphorus, an increased clearance of phosphorus, and a lower concentration of phosphorus in the serum. These data do not permit the conclusion that probenecid therapy induces a phosphaturic response in hypoparathyroid subjects by interfering with the renal tubular absorption of phosphorus.

THE urinary excretion of phosphorus in euparathyroid subjects is not influenced by probenecid (1, 2). However, recent investigators have proposed that the renal response to a potentially phosphaturic agent could be masked by the regulatory effect of circulating parathyroid hormone. This contention has been supported by the demonstration that prolonged treatment of hypoparathyroid subjects with probenecid is accompanied by a reduction of hyperphosphatemia (3). However, the proposal that this effect is the consequence of an increased phosphaturia has not been substantiated by the determination of specific renal functions. In the following study, specific renal functions were examined in 4 patients with hypoparathyroidism, before and after the intravenous administration of probenecid. No phosphaturic response was observed.

METHODS

Three adult females with postoperative hypoparathyroidism and 1 with idiopathic hypoparathyroidism were studied (see Appendix). Renal clearances were determined by standard techniques. A priming dose of inulin was administered intravenously and its concentration in the serum was maintained by continuous infusion at a constant rate. After an equilibration period of one hour, urine was collected by catheter during 3 consecutive periods of approximately twenty minutes' duration. At the end of the third collection period, a 2-Gm. dose of probenecid was administered intravenously in 150 ml. of

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0.9 per cent saline during a 30-minute period, after which 3 additional urine samples were collected. Blood was collected by venipuncture for the determination of inulin (4), phosphorus (5), and *p*-aminohippurate (PAH) (6). When PAH was used, a priming dose was administered intravenously at the end of the third collection period, and the concentration of PAH in the plasma was maintained by continuous infusion. Urine collections were continued after a 45-minute equilibration period. No exogenous phosphate was administered in any of the experiments.

RESULTS AND DISCUSSION

The experimental conditions were tested to determine whether they were adequate for the demonstration of a phosphaturic response to probenecid therapy. First, *p*-aminohippurate (PAH) was used as a phosphaturic agent (7) instead of probenecid. Phosphaturia was observed in each of 3 subjects and was reflected in a significant decrease in the serum concentration of phosphorus (Table 1 a). A second test was made by observing the influence of probenecid on the maximal renal excretory rate for PAH (TmPAH). In Patient MH, the TmPAH was reduced from 53 mg. per minute to 6 mg. per minute. Since the sequences and time intervals in the tests were identical to those proposed for the experimental observations, the experimental design was considered adequate for the demonstration of a phosphaturic response to the administration of probenecid. The results obtained with probenecid are summarized in Table 1 b. No increase in phosphorus excretion was elicited in any of the 4 patients, nor did the serum concentration of phosphorus change. The recorded decreases in urinary excretion of phosphorus are not significant.

These results contrast with published opinions. Pascale, Dubin and Hoffman (3) demonstrated that prolonged therapy with probenecid reduced the hyperphosphatemia of 1 pseudohypoparathyroid and 4 hypoparathyroid subjects. This was interpreted as resulting from increased phosphaturia, although the data on phosphate excretion (which were recorded for only 2 of the 5 subjects) do not support this contention unequivocally. The justification for comparing the acute studies of this report with the prolonged studies of Pascale *et al.* is their claim that hypophosphatemia, and by implication phosphaturia, occurred promptly after therapy was initiated, although it was not always maintained during the later stages of the experiment. Thus, if phosphaturia increased in the first twenty-four hours of oral therapy, a similar response would be expected shortly after the intravenous administration of probenecid.

Additional studies were presented by Dubin *et al.* (8), who concluded that probenecid increased the 24-hour renal clearance of phosphorus in hypoparathyroid subjects. The recorded data barely support the argument and might be considered unreliable because of the large errors inherent in the measurement of the 24-hour clearances of inorganic ions. Baer

TABLE 1(a). EFFECT OF PAH ON PHOSPHORUS (P) EXCRETION*

Patient		GFR (ml./min.)	Excretion of P (μg./min.)	Plasma P (μg./ml.)	Plasma PAH (mg./100 ml.)
EP	Control	71.5	340	70	0
	Exper.	59.5	1010	65	46
AP	Control	80.5	150	59	0
	Exper.	74.2	760	49	69
MH	Control	55.9	440	73	0
	Exper.	52.3	810	67	17

TABLE 1(b). EFFECT OF PROBENECID ON PHOSPHORUS (P) EXCRETION*

Patient		GFR (ml./min.)	Excretion of P (μg./min.)	Plama P (μg./ml.)
EP	Control	68.2	80	66
	Exper.	69.6	70	65
AP	Control	84.8	310	62
	Exper.	90.4	40	59
MH	Control	66.7	320	58
	Exper.	62.9	250	57
DA	Control	87.0	130	43
	Exper.	81.6	30	42

* Each figure represents the average of 3 observations.

et al. (9) observed increased phosphaturia in response to probenecid therapy in 1 of 2 patients with pseudohypoparathyroidism. However, the results were variable and transitory and could not be reproduced. Another approach was taken by Kolb and Rukes (10), who indicated that probenecid increased the fecal excretion of phosphorus in gouty subjects and caused a prompt phosphate diuresis in hypoparathyroid subjects. Since this observation was reported only in abstract form, no data are available for critical analysis. Furthermore, the experience of Ford and Spurr (11) casts doubt on the validity even of these comments. These authors reported that probenecid increased the fecal excretion of phosphate in 5 gouty subjects without influencing the urinary excretion. When the program was enlarged to include 7 control subjects and 7 patients with gout, Spurr, Ford

and Moyer (12) concluded that probenecid did not alter significantly either the urinary or the fecal excretion of phosphorus. Schneider and Corcoran (13) produced a depression of the renal tubular absorption of phosphorus with probenecid in a subject with an obscure renal lesion, but insufficient data were offered and the magnitude of the effect cannot be assessed. None of these observations provides a definitive demonstration of the phosphaturic effect of probenecid. In contrast to these reports, Jackson *et al.* (14) were unable to alter the phosphaturia either in a hypoparathyroid or in a pseudohypoparathyroid subject. Similarly, the present results do not demonstrate a phosphaturic response to probenecid during acute renal clearance measurements in 4 hypoparathyroid subjects. Consequently, on the basis of currently available information, it is not permissible to conclude that probenecid interferes directly with the renal tubular absorption of phosphorus.

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APPENDIX

DA was a 42-year-old white woman in whom tetany, myxedema and laryngeal nerve paralysis developed following a thyroidectomy in 1952. All medications were discontinued on January 21, 1957. On February 4, 1957 she exhibited signs of hypocalcemia, necessitating therapy with oral calcium gluconate. Clearances were estimated on February 12. On February 16, 1957, the serum concentration of calcium was 8.3 mg. per 100 ml.

AP was a 64-year-old diabetic white woman in whom tetany developed two months following a thyroidectomy in 1954. Sterol therapy was discontinued on March 10, 1955 in preparation for estimation of phosphorus clearance on April 28 and May 6. During hospitalization she was treated with 3 grains of thyroid, 30 units of insulin, and 4 Gm. of calcium lactate daily. On April 27, 1955, the serum concentration of calcium was 6.0 mg. per 100 ml.

EP was a 62-year-old white woman with a 30-year history of grand mal epilepsy, in whom a diagnosis of idiopathic hypoparathyroidism was established in 1952. Sterol therapy was discontinued on March 18, 1956, prior to phosphorus clearance determinations made on May 16 and 21, 1956. During this period, the serum concentration of calcium averaged 6.8 mg. per 100 ml.

MH was a 37-year-old woman in whom tetany and hypoparathyroidism developed following a thyroidectomy in 1943. Sterol therapy was discontinued on January 4, 1957, in preparation for phosphorus clearance determinations on February 6, 1957, but treatment with thyroid and oral calcium lactate was continued. On February 4, the concentration of serum calcium was 7.2 mg. per 100 ml. Clearances had also been performed in 1955, at which time the serum concentration of calcium was 9.0 mg. and of phosphorus 7.6 mg. per 100 ml.

A MICRO-METHOD FOR THE DETERMINATION OF BUTYL-ALCOHOL EXTRACTABLE HORMONAL IODINE IN SERUM*†

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ABSTRACT

A micromethod for the determination of serum butanol-extractable hormonal iodine, using an ashing technique and adapted to the Beckman DU spectrophotometer, is described. The adaptation of the method to this spectrophotometer has been achieved by employing the inhibitory effect of Ag^+ on iodide catalysis of the ceric-arsenious reaction.

Chemical kinetics pertaining to the catalytic effect of I^- on the ceric-arsenious reaction are analyzed and discussed. Under the conditions of this procedure, the catalytic effect of I^- on the ceric-arsenious oxidation-reduction reaction follows a first-order rate, and therefore a linear calibration curve can be constructed relating the

$$\text{logarithmic ratio } \frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube (no } I^-)}$$

to the concentration of added I^-.

Recoveries of freshly prepared thyroxine alone and thyroxine added to serum, using the prescribed procedure, ranged from 92 to 101 per cent.

This method effectively eliminates potential contaminants of serum such as mono- and diiodotyrosine, inorganic iodide, and heavy metal.

INTRODUCTION

AN ACCURATE determination of serum "thyroxine-like" iodide is a reliable laboratory aid in the evaluation of thyroid function and is particularly important in the pediatric patient (1–3).

It is the purpose of this communication to present a method for determining hormonal iodide in small quantities of serum, which is accurate and may be adapted to the research or clinical laboratory. This method is based upon: (a) butyl-alcohol extraction of iodinated amino-acids and iodide from serum, (b) separation of thyroxine-like compounds from other

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iodinated amino-acids or iodide by alkali wash, (c) evaporation and ashing of the alkali-washed extract, and (d) estimation of iodide by its catalytic effect on the ceric-arsenious oxidation-reduction reaction. Critical to the latter has been adaption of the determination to the Beckman DU spectrophotometer made possible through a method for selectively stopping the catalytic effect of iodide on the ceric-arsenious reaction.

Data are presented which pertain to chemical kinetics, and each phase of the procedure is evaluated.

EXPERIMENTAL

Reagents

10 per cent H_2SO_4 by volume (4).

4 *N* NaOH in 5 per cent Na_2CO_3 by volume (5, 6).

4 *N* Na_2CO_3 solution.

10 per cent KNO_3 by volume. (KNO_3 should be purified by recrystallization.)

0.3 *N* $H_2AsO_3^-$ (oxidation concentration). This solution is prepared by adding 14.84 Gm. of As_2O_3 (Mallinckrodt's primary standard reagent) to about 500 ml. of demineralized water in a 1000-ml. beaker. To this, 28 ml. of concentrated H_2SO_4 are added and the contents of the beaker heated on a hot plate until the As_2O_3 is dissolved. The resulting solution is cooled, transferred to a 1-liter volumetric flask and brought to 1000 ml. with demineralized water. A small piece of metallic arsenic is added to this solution as recommended by Man (7).

0.1 *N* $Ce(HSO_4)_4$ (reduction concentration). This stock solution is prepared by dissolving 52.8 Gm. of $Ce(HSO_4)_4$, anhydrous, in a 1-liter volumetric flask with 5 *N* H_2SO_4. The mixture is warmed in a hot-water bath, with occasional mixing, until a clear solution is obtained (approximately one hour) and then diluted to the mark with 5 *N* H_2SO_4. The oxidation-reduction concentration of this solution is established by standardizing it with the 0.3 *N* $H_2AsO_3^-$ solution. The ratio of the titration volumes of $Ce(HSO_4)_4$ solution to 0.3 *N* $H_2AsO_3^-$ solution in the presence of I^- should be 3:1 (7).

0.02 *N* Ce^{++++} solution. This solution is made from the 0.1 *N* Ce^{++++} solution by diluting it with 5 *N* H_2SO_4.

Arsenious acid mixture (for dissolving the contents of the ashed tube). This is freshly prepared by mixing 2.5 *N* H_2SO_4, 0.3 *N* $H_2AsO_3^-$ and ion-free water in a volume ratio of 1:1:2, respectively.

0.01 per cent $AgNO_3$ by volume.

Analytical Procedure

Step 1—Serum n-butyl alcohol extraction and washing

To 0.50 ml. of serum, or less, in a glass-stoppered tube, 0.2 ml. of 10 per cent H_2SO_4 is added, mixed, and followed by 3 ml. of *n*-butyl alcohol. The mixture is shaken for thirty seconds and then centrifuged at 2000 rpm for ten minutes at room temperature. After centrifugation the butyl alcohol is transferred to a separatory funnel. This process of extraction is repeated twice, using 2 ml. of butyl alcohol per extraction and shaking the tube for twenty seconds after the addition of the butyl alcohol. Centrifugation is carried out at 2000 rpm for seven to eight minutes for the second extraction and fifteen minutes for the last extraction.

The combined butyl-alcohol extract is washed in the separatory funnel with 7 ml. of

4 *N* NaOH in 5 per cent Na_2CO_3 by shaking the funnel for twenty seconds and letting it stand (about half an hour) until complete separation of phases occurs. The alkali phase is removed and another similar washing is performed. After a third similar washing, the mixture is transferred to a Pyrex test tube (18×150 mm.). The butyl-alcohol phase is separated by centrifugation at 2000 rpm for twenty minutes at 0–5° C. Following centrifugation, the washed butyl-alcohol extract is transferred to another Pyrex test tube (18×150 mm.) containing 0.50 ml. of 4 *N* Na_2CO_3 and 0.1 ml. of 10 per cent KNO_3.

Step 2—Evaporation and ashing of washed butyl-alcohol extract

The tube is placed at a 45° angle in a sand bath on top of a hot plate; infrared lamps are focused on the tube from above and below. During evaporation the temperature of the sand bath approximates 120° C. This process is carried out in a hood and requires approximately two hours. The dried, extracted specimen is then ashed in a muffle furnace at 520° C. for four hours.

Step 3—Final estimation of I^-

The ashed material in the tube is dissolved in 5.0 ml. of the arsenious-acid mixture, and the solution is then centrifuged at 2000 rpm for twenty minutes at room temperature. Following centrifugation, a 4.0-ml. aliquot is transferred to another Pyrex tube of the same size and placed in a water bath at 30° C. until temperature equilibrium is attained (about fifteen minutes). At zero time, 1.0 ml. of 0.02 *N* Ce^{++++} (temperature equilibrated) is added and the contents of the tube mixed with a stirring rod. After exactly twenty minutes' incubation the reaction is stopped by the addition of 1 drop (approximately 0.05 ml.) of 0.01 per cent $AgNO_3$. The optical density (O.D.) of the solution is read at 430 mμ in the Beckman DU spectrophotometer, the ratio of

$$\frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube (no } I^-)}$$

is obtained, and the butyl-alcohol extractable hormonal iodide (BEI) content of the sample is calculated from the standard curve (see construction of standard curve).

The contents of the control tube are processed in the same manner as the serum sample except that 0.5 ml. of water instead of serum is added to the glass-stoppered tube.

Construction of standard curve

A standard curve for estimating the serum BEI value is constructed using KI (standard solution) in concentrations covering the range from 0 to 0.08 μg. of I^- per tube (O.D. from 1.0 to 0.25, respectively), which corresponds to a maximum BEI value of 20 μg. of I^- per 100 ml. of serum when 0.50 ml. of serum is used. The procedure under Step 1 is employed to obtain alkali-washed portions of butanol to which are then added proper amounts (ranging from 10 to 100 μl.) of a carefully prepared KI solution (1.00 μg. of I^- per ml.). Control tubes containing no I^- are made up by the same procedure. Thereafter steps 2 and 3 are carried out. The ratio of the O.D. of each standard I^--containing tube to the O.D. of the control tube (no I^- added) is obtained and plotted on a semi-log scale versus the corresponding I^- concentration. Since the ceric-arsenious oxidation-reduction reaction in the presence of I^- follows a first-order rate, a linear relationship should be obtained (see kinetics). A representative standard curve is presented in Figure 1.

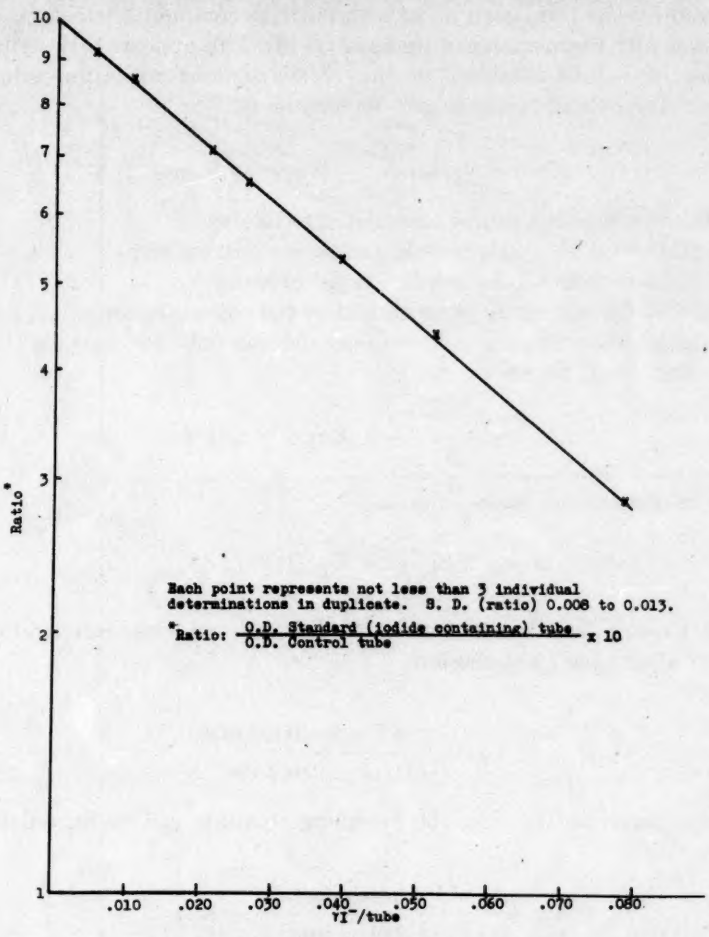


FIG. 1. Final I^- standard curve for BEI method.

EVALUATION OF THE METHOD

Kinetics of the Catalytic Effect of Iodide on the Ceric-Arsenious Oxidation-Reduction Reaction

1. *Order of reaction*

Analysis of the reaction rate was considered critical to the establishment of a standard technique for the final calculation of iodide concentration. Homogenous catalytic processes in solution appear to be generally char-

acterized by the formation of an intermediate compound which then decomposes with regeneration of the catalyst (8). This appears to describe the kinetics of iodide catalysis in the ceric-arsenious oxidation-reduction process. The overall reaction may be written as:

$$2Ce^{++++} + H_2AsO_3^- \xrightarrow{I^-} 2Ce^{+++} + H_3AsO_4.$$

Stepwise this reaction can be formulated as follows:

[1] $2Ce^{++++}+2I^- \rightarrow 2Ce^{+++}+I_2$ (rate-determining step)

[2] $H_2AsO_3^-+I_2 \rightarrow H_3AsO_4+2I^-$ (rapid process).

The velocity is directly proportional to the concentration of I^-, so that the rate-determining step involves one such ion only and may be $Ce^{++++}+I^+=Ce^{+++}+I$; therefore

$$\frac{-d(Ce^{++++})}{dt} = K\,(Ce^{++++})\,(I^-).$$

Upon integration we have

$$1n\,\frac{Co}{C} = K(t)(I^-)$$

where Co = original concentration of Ce^{++++} and C = concentration of Ce^{++++} after time t has elapsed.

Since

$$\log \frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube}}$$

is proportional to 1nC/Co, the foregoing equation can be formulated as follows:

$$\log \frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube}} = K'(I^-).$$

By plotting

$$\log \frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube}}$$

versus I^- concentration or versus time, a linear relationship should result if the reaction follows a first-order rate. Such a relationship of time or concentration of iodide to

$$\log \frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube}}$$

was found to exist under the conditions of the studies represented in Figure 2.

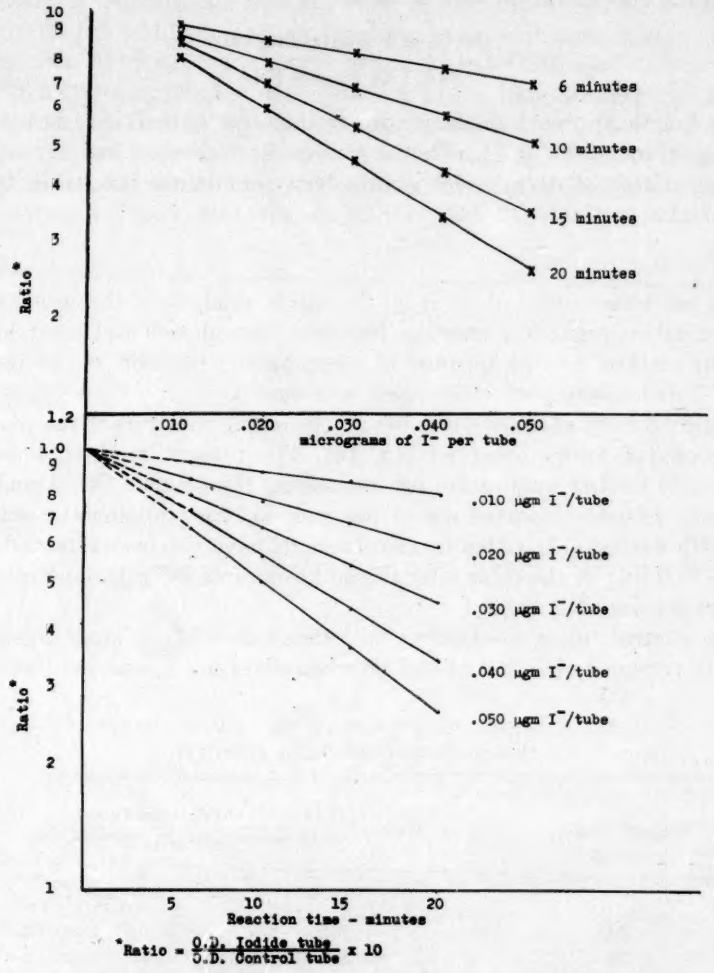


FIG. 2. Iodide catalysis of ceric-arsenious reaction.

The experiment was carried out according to the technique for the final estimation of iodide already presented (see Analytical Procedure), with the following exceptions:

(a) Each tube contained 1.0 ml. of 0.3 *N* arsenious acid, 1.0 ml. of the iodide standard, and 2.0 ml. of water. After temperature equilibration of reactants, the reaction was initiated by addition of 1.0 ml. of 0.02 *N* ceric solution (temperature equilibrated).

(b) At the times indicated, the reaction was stopped by the addition of silver ion.

From these studies we have concluded that the reaction, under the conditions employed, follows a first-order rate. Therefore it is logical to plot the iodide concentration versus the

$$\log \frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube}}$$

The kinetic approach to elucidation of the order of reaction has been investigated by others (9–11). The present studies provide a logical basis for the calculation of data, which satisfactorily eliminates the necessity for less reliable methods (12–14).

2. *Effect of silver ion*

The inhibitory effect of silver on the iodide catalysis of the ceric-arsenious oxidation-reduction reaction has been introduced and used in the present method for the purpose of stopping the reaction at the desired time. This inhibitory effect is shown in Figure 3.

The inhibitory effect of silver ion on the iodide catalysis of the reaction has been previously observed (15, 16). The present method, however, represents its first application for controlling the process. Strickland and Maloney recently reported use of mercuric ion for stopping the reaction in a PBI method (17). Other means of control have also been reported (14).

The stability of the color after the addition of silver is demonstrated by studies presented in Table 1.

The control tubes (containing no iodide) showed a change in optical density per hour of only 0.04 to 0.06 when silver ion was added (before or

TABLE 1. EFFECT OF SILVER ON IODIDE CATALYSIS OF CERIC-ARSENIOUS REACTION-COLOR STABILITY

Elapsed* time (mins.)	Ratio: $\frac{\text{O.D. of } I^-\text{-containing tube}}{\text{O.D. of control tube}}$	
	0.05γ I^-/Tube	0.02γ I^-/Tube
20	.261	.588
30	.260	
40	.262	.588
60	.263	.590
100	.266	.585
120	.266	.587

* The time elapsed between the addition of silver ion and the O.D. determination. Silver ion was added after the reaction had been run at 30° C. (water bath) for twenty minutes. Ceric reagent and arsenious acid had been added as described in the method section.

after incubation); whereas, without silver ion, the change was greater than 0.20.

These data demonstrate that the color is stable for at least a two-hour period after the addition of silver. The advantages inherent in controlling the reaction rate with the addition of silver are as follows:

(a) It makes possible the determination of the light absorbancy of the remaining ceric ion after an exact incubation period.

(b) It permits adaptation of the method to the standard DU Beckman spectrophotometer without special temperature control equipment or the rate-determining devices of Chaney (16).

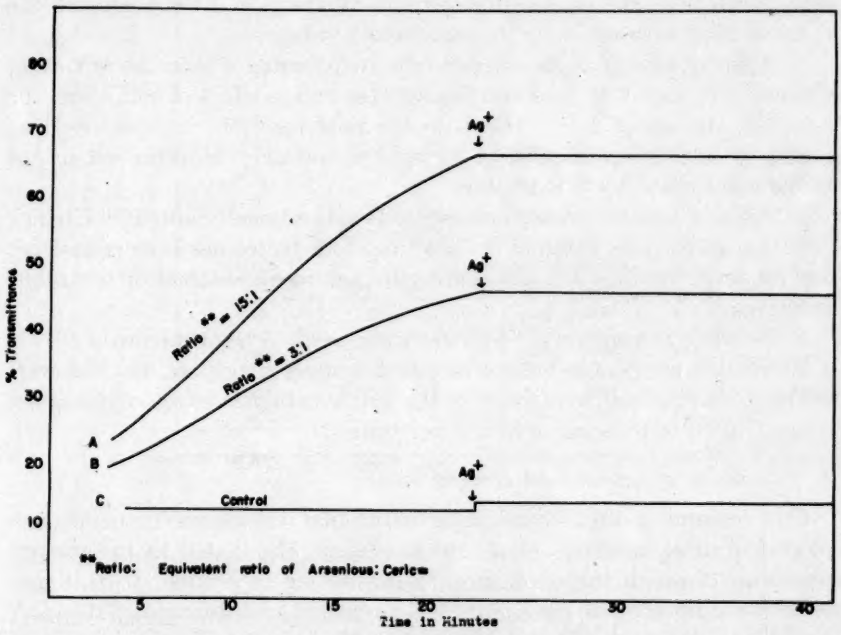


FIG. 3. Effect of silver ion on iodide catalysis of ceric-arsenious reaction.*

Reaction vessels for this experiment contained:

A. 0.50 ml. of 0.3 *N* arsenious acid+0.100 γ of iodide and 0.50 ml. of 0.02 *N* ceric sulfate diluted to 5.0 ml. total volume with demineralized water.

B. Same as A, except the equivalent ratio of arsenious acid to ceric sulfate was 3:1; 0.50 ml. of 0.06 *N* arsenious acid instead of 0.3 *N* arsenious acid was used.

C. Same as A but no iodide was added.

In each experiment an aliquot from each vessel was transferred to a corex cell and the reaction rate observed on the Beckman DU recording spectrophotometer at 420 mμ and at 20° C. At the time indicated, 0.05 ml. (1 drop) of 0.01 per cent silver nitrate solution was added.

(c) It facilitates ease of manipulation of individual samples and increases the number of samples which one technician can accurately process.

3. *Selection of optimal conditions*

Investigations were conducted to determine the optimal concentrations of chemical constituents in the reaction, and optimal conditions for time, temperature and wave length.

a. *Arsenious-ceric equivalent ratio.* The influence of the equivalent ratio of arsenious acid to ceric ion on the reaction rate has been investigated by Lein and O'Neal (13, 18). They observed that increases in the concentration of arsenious acid above the equivalent ratio of 10:1 did not alter the rate of reaction. We have confirmed these findings and have selected the ratio of 15:1 with consistently satisfactory results.

b. *Effect of acidity on the reaction rate.* In accordance with the reports of Chaney (16) and of O'Neal and Simms (13) on the effect of acidity on the reaction, the use of 1.4 *N* H_2SO_4 in the final reaction mixture has been found to insure reproducibility of light absorbancy of ceric ion in the range determined by this method.

c. *Effect of sodium ion on the reaction.* It was originally noted by Chaney (16) that sodium ion inhibits the reaction. This factor has been considered and rendered minimal and constant by the ashing and extraction technique described.

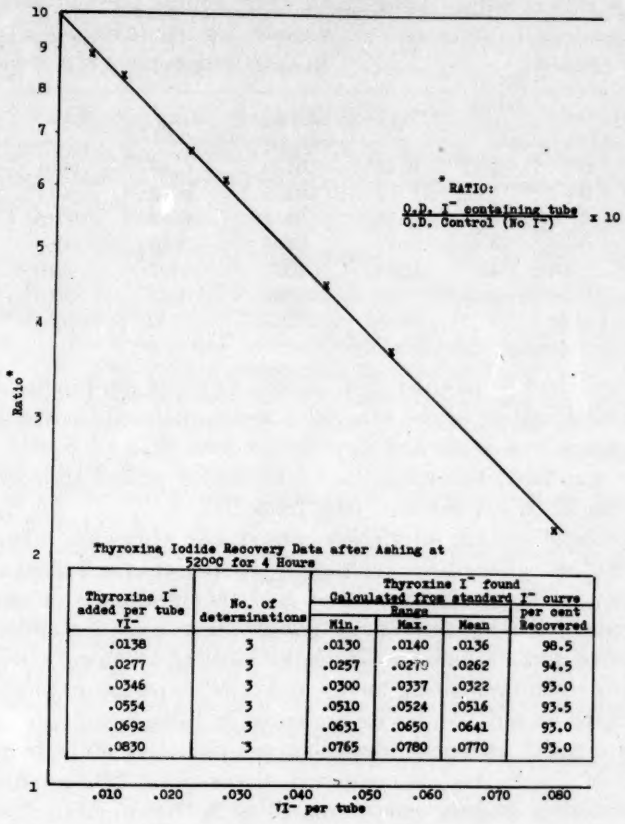
d. *Selection of temperature, time and wave length.* A temperature of 30° C., a 20-minute period for incubation, and a wave length of 430 mμ were selected for maximal sensitivity of the iodide catalytic effect covering the range from 0 to 0.08 μg. of iodide per tube.

4. *Evaluation of the ashing technique*

Cumbersome and time-consuming distillation techniques frequently employed in other methods (4, 7, 19) have been eliminated in the present procedure through the application of an ashing technique. In this procedure, modified from that proposed by Salter and Johnston (20), 4 *N* Na_2CO_3 and recrystallized KNO_3 are used. Recrystallization of KNO_3 eliminates contaminants which affect the reaction. Ashing is conducted at 520° C. for four hours. Experiments establishing the validity of this ashing technique are presented in Figure 4.

The points on the graph in Figure 4 are a plot of values derived from standard solutions of KI carried through the ashing technique and final determination. The table at the bottom of this figure presents data on the recovery of thyroxine iodide[1] carried through ashing and final determination. Thyroxine iodide recoveries ranged from 93 to 98.5 per cent.

Recovery of iodide or thyroxine iodide added to alkaline-washed butyl



Thyroxine Iodide Recovery Data after Ashing at 520°C for 4 Hours

Thyroxine I^- added per tube YI⁻	No. of determinations	Thyroxine I^- found Calculated from standard I^- curve Range Min.	Max.	Mean	per cent Recovered
.0138	3	.0130	.0140	.0136	98.5
.0277	3	.0257	.0270	.0262	94.5
.0346	3	.0300	.0337	.0322	93.0
.0554	3	.0510	.0524	.0516	93.5
.0692	3	.0631	.0650	.0641	93.0
.0830	3	.0765	.0780	.0770	93.0

FIG. 4. Evaluation of ashing technique.

alcohol and ashed was as good as that shown in Figure 4 for iodide and thyroxine iodide only carried through ashing. It is at this point in the procedure that iodide or thyroxine iodide is normally introduced for construction of standard curves. Such an iodide standard curve has already been presented in Figure 1.

5. *Evaluation of the butyl-alcohol extraction procedure*

The butyl-alcohol extraction of iodinated amino-acids from biologic fluids and tissues was originally employed by Leland and Foster, adapted by Chaikoff, and is currently recognized as a technique of decided ad-

[1] Crystalline sodium-*l*-thyroxine obtained from Aldrich Chemical Company, Milwaukee, Wisconsin. Recovery calculations are based on the theoretical iodide content of sodium-thyroxine.

TABLE 2. THYROXINE RECOVERY DATA

Thyroxine added (γ I^-/tube)	Pooled serum (ml.)	No. of det'ns	Range (γ I^-/tube)				% Recovery
			Min.	Max.	Mean	S.D.	
.0145	0	3	.0142	.0150	.0146	.0004	101
.0290	0	3	.0269	.0288	.0282	.0011	97.5
.0580	0	3	.0516	.0544	.0534	.0015	92.0
.0725	0	3	.0610	.0735	.0675	.0060	93.0
0	.50	4	.0299	.0321	.0307	.0010	—
.0250	.50	3	.0521	.0552	.0530	.0016	96.0
.0500	.50	3	.0738	.0769	.0752	.0016	94.0

vantage over the PBI method (2, 6, 21, 22). In the present method we have applied a modification of the extraction technique used by Man *et al.* (4). The procedure was evaluated directly by recoveries of *freshly* prepared thyroxine standards following their addition to pooled sera. Recoveries ranged from 92 to 101 per cent (see Table 2).

The effects of certain interfering compounds which are potential contaminants of sera, have been evaluated. The present extraction and washing procedure effectively eliminates such contaminants as mono- and diiodotyrosine, iodide, and heavy metal when they are added in large quantities to sera. Man and associates have also shown a satisfactory elimination of contaminating mono- and diiodotyrosine and iodide (2, 4). Data from the present studies are presented in Table 3.

The elimination of such interfering compounds greatly increases the reliability of the method as compared to the usual PBI methods (2, 4).

The present procedure was compared with that of Man *et al.* by determination of BEI values on paired serum specimens from Man's laboratory. There was excellent agreement on all specimens analyzed (values ranged from 1.4 to 8.8 μg. per 100 ml.). As a result of these studies we have adopted Man's standards for normal BEI concentration in the serum of

TABLE 3. EFFECT OF INTERFERING COMPOUNDS ON BEI VALUES

No. of det'ns	Pooled serum (ml.)	Interfering compound	Range (μg. I^-/100 ml.)		
			Min.	Max.	Mean
4	.50	None	5.98	6.42	6.14
3	.50	.650 γ I^- (as KI)	5.70	6.12	5.97
3	.50	2.40 γ I^- (as mono- and diiodotyrosine sol'n)	5.56	6.50	5.91
3	.50	3.0 γ Ag^+ (as $AgNO_3$)	6.20	6.50	6.30

infants, children and adults (2, 3). During the past year this method has been used experimentally and clinically in several hundred determinations with consistently good results (23).

Acknowledgment

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Letters to the Editor

EFFECT OF AN ALDOSTERONE ANTAGONIST ON SODIUM AND POTASSIUM EXCRETION IN PRIMARY HYPERALDOSTERONISM

To the Editor:

Recent observations of Kagawa and associates (1) on adrenalectomized rats, and of Liddle (2) on human beings suggest that the synthetic steroid, 3-(3-oxo-17β-hydroxy-4-androsten-17α-yl)-propionic acid γ-lactone (SC-5233), and its 19-nor analog (SC-8109) can block the effects of aldosterone or of desoxycorticosterone. Recently we have had the opportunity to study the effects of one of these new compounds, SC-8109,[1] in a patient with primary hyperaldosteronism.

A 49-year-old woman was found to have alkaline urine of low specific gravity, hypokaliemic alkalosis, electrocardiographic evidence of hypokaliemia and severe hypertension. During the entire study, the patient received a constant diet. Each metabolic period consisted of six days, except the fifth period which was seven days in length. Urinary aldosterone was determined by a modification of the method of Neher and Wettstein (3), and methods previously described (4) were used for other determinations.

As shown in Figure 1 the sodium and potassium balances were slightly positive during the first 2 periods and the daily sodium-potassium ratio in the urine was essentially the same as in the intake. Urinary aldosterone excretions on 2 occasions during these periods were 32 and 56 μg. per day. Oral administration of 1 Gm. of SC-8109 daily during the third period resulted in mild but definite changes in sodium and potassium balances and in the urinary sodium-potassium ratio. The administration of 1.9 Gm. of SC-8109 daily during the fourth and fifth periods resulted in further retention of potassium, a strongly negative sodium balance, a threefold increase in the urinary sodium-potassium ratio, a decrease in plasma carbon dioxide content, an increase in plasma potassium concentration and a decrease in the ratio of exchangeable sodium and potassium in the presence of continued elevation of aldosterone in the urine. The strongly negative sodium

[1] SC-8109 was obtained through the courtesy of Dr. Clarence L. Gantt of Searle & Co., Chicago, Illinois.

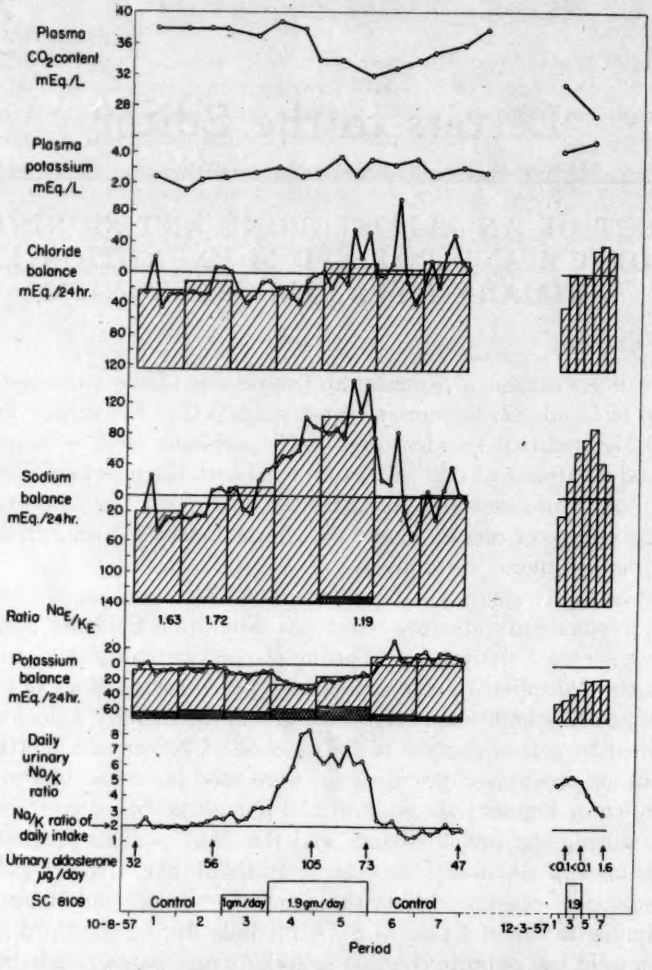

FIG. 1. Effect of SC-8109 on electrolyte metabolism in a patient with primary hyperaldosteronism. The balance data for chloride, sodium and potassium are charted so that a negative balance is represented by extension of the column above the zero line and a positive balance by a clear space below the zero line.

balance was not associated with a comparable change in chloride balance and there was no significant change in total body water (30.28 liters as compared to 30.76 liters). Excretion of aldosterone in the urine increased significantly in these periods.

When the use of SC-8109 was discontinued (periods 6 and 7) the urinary

sodium-potassium ratio rapidly fell to control values, the potassium balance became negative and the carbon dioxide content of the plasma increased. There was no change in hypertension during the administration of SC-8109 and the clearance of endogenous creatinine remained unchanged.

Six days after the removal of an adrenocortical adenoma that weighed 45 Gm., the preoperative diet was resumed and the daily urinary excretion of sodium, potassium and chloride was determined for seven days. The oral administration of 1.9 Gm. of SC-8109 per day on the third and fourth days had little effect on the high urinary sodium-potassium ratio. There was no measurable aldosterone in the urine (<0.1 μg. per day) on the second and fourth days, and the sodium-potassium ratio of the urine was approximately 2.5 times that of the intake. The urinary sodium-potassium ratio fell on the fifth, sixth and seventh days and aldosterone was detectable in the urine on the seventh day.

Compound SC-8109 produced a marked effect on the excretion of sodium and potassium when a large amount of aldosterone was being excreted; it produced a negligible effect when only a trace of aldosterone was being excreted. These results support the conclusions of Kagawa and associates (1), and of Liddle (2) that this synthetic steroid acts as an antagonist of aldosterone.

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A C_{21}-DESOXYSTEROID WITH CORTICOID ACTIVITY

TO THE EDITOR:

A C_{21}-oxygen function has been thought to be essential for the development of a steroid with cortisone-like activity. The biosynthesis of C_{21}-oxygenated steroids proceeds only in the adrenal gland and this type of steroid has not been isolated after ingestion of large amounts of C_{21}-desoxycortisone (1) and C_{21}-desoxyhydrocortisone (2). It was therefore of interest to observe that a synthetic C_{21}-desoxysteroid, $\Delta^{1,4}$-pregnene-9α-fluoro,6-methyl,11β,17α-diol-3,20-dione (Upjohn-8614) was a potent corticoid.

U-8614 given by mouth to 5 patients with normal adrenal function, at a level of 15 to 20 mg. a day in fractional doses, suppressed adrenocortical secretion (Table 1). Corticoids in the plasma and urine were measured by a modification of the Silber-Porter technique (3), and urinary 17-ketosteroids by the method of Werbin and Ong (4). In all but 1 patient (O.H.), plasma and urinary corticoids approached zero during the four- to six-day period of administration of U-8614. Suppression of urinary corticoids was apparent

TABLE 1. EFFECT OF $\Delta^{1,4}$-PREGNENE-9α-FLUORO,6-METHYL, 11β,17α-DIOL-3,20-DIONE ON ADRENAL FUNCTION

Patient	Dose of U-8614 (mg./24 hrs.)	Urine 17-OHCS (mg./24 hrs.)		Plasma 17-OHCS (μg./100 cc.)		Urine 17-KS (mg./24 hrs.)	
		Control*	Drug†	Control*	Drug†	Control*	Drug†
N.M., ♀ 47, Ca. of breast	15	5.5	0	21	0.8	4.7	0.7
	45	5.3	0	19	0	4.9	1.8
A.M., ♀ 32, Ca. of breast	15	9.2	0.7	26	3.9	5.4	1.2
L.F., ♂ 58, Ca. of prostate	15	7.5	1.8	17	3.6	4.1	2.2
M.S. ♂ 68, Ca. of prostate	15	3.1	0.7	18	4.5	5.7	4.1
N.A. ♀ 48, Ca. of breast	20	8.2	1.4	11	0	3.9	2.2
O.H., ♂ 55, Adrenal hyperplasia	20	16.1	6.7	28	19	8.3	3.7

* Average value for period of 4 to 6 days.
† Lowest value obtained.

on the first day of the test period. When administration of U-8614 was stopped, the urinary corticoids returned to normal within a 24-hour period in 4 of the patients, and after four days in the fifth patient. Subject O.H., a woman with Cushing's syndrome due to adrenal hyperplasia, received U-8614 in a dosage of 20 mg. a day for six days. This resulted in some suppression of both urinary corticoids and 17-ketosteroids. The decrease in these parameters of adrenal function was not as profound as that seen with 8 mg. of Δ^1,9α-fluorohydrocortisone.

The urinary 17-ketosteroids were likewise suppressed by U-8614. As conversion of C_{21}-desoxyhydrocortisone to 17-ketosteroids in approximately 10 per cent yield has been demonstrated (5), it is possible that metabolites of U-8614 comprise part of the measured 17-ketosteroids. More detailed studies of the urinary 17-ketosteroids are in progress.

These data strongly suggested that U-8614 inhibited the production or release of corticotropin (ACTH). In order to determine whether U-8614 was affecting adrenal function at the level of the adrenal gland, an intravenous ACTH test was performed on a patient receiving U-8614. The response of the plasma corticoid level to the ACTH was normal and of the same magnitude as in a subsequent test performed on the same patient during a control period.

In order to explore other indices of corticoid activity, U-8614 was given to 2 hypophysectomized patients who were in a state of adrenal insufficiency following withdrawal of maintenance cortisone. U-8614, in a dosage of 15 mg. a day, led to restoration of the eucorticoid state within twenty-four hours. Water tolerance was restored to normal in 1 patient.

From these data we have concluded that U-8614 possesses many of the activities of hydrocortisone and is somewhat more potent than hydrocortisone. The activity of this compound, lacking what was previously assumed to be an essential functional group, may point the way towards even more selective modifications of the steroids.

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DETECTION OF ANTITHYROID ANTIBODIES

TO THE EDITOR:

Witebsky *et al.* (1, 2) recently described the occurrence of antibody to a component of human thyroid extract in the serum of patients with chronic thyroiditis, and Roitt, Doniach and others (3, 4) reported similar findings on patients with Hashimoto's disease. Witebsky and coworkers have particularly employed the tanned red-cell hemagglutination method of Boyden for demonstration of the antithyroid antibody, and the English workers used the agar-gel diffusion method of Ouchterlony. Preliminary studies using Coons' labeled antibody staining method (5) suggest that it can be used as a qualitative test for antithyroid antibody. Frozen sections of unfixed normal human thyroid were treated for one hour with sera from 3 patients with a clinical history suggestive of chronic thyroiditis, or with control sera. The slides were then washed, treated for ten minutes with fluorescein-labeled rabbit anti-human gamma globulin, and examined under ultraviolet light. Two of the 3 test sera induced distinctly enhanced staining of thyroid-cell cytoplasm as compared to the control sera, indicating that antibodies in these sera had been bound by cytoplasmic proteins. No definite staining of colloid was observed—a finding of interest in view of the possibility that the antibody in question reacts with thyroglobulin (1, 3). Staining of cells or colloid was not observed when these same sera were tested on sections of normal rabbit thyroid. That the material deposited from the active sera was actually gamma globulin is shown by the fact that no stain was observed when sections were treated with unlabeled rabbit anti-human gamma globulin, before staining with fluorescein-labeled rabbit antibody.

Although the Coons method as usually employed does not provide quantitative information, its simplicity suggests that it may be of value as a diagnostic aid, for detection of antithyroid antibody. For example, the third patient mentioned here, whose serum apparently did not contain

antibody, has been shown by additional tests of thyroid function to have colloid goiter. The 2 patients with antithyroid antibody have clinical histories suggesting an episode of thyroiditis, followed by hypothyroidism, but have not undergone a biopsy procedure, so that the exact nature of their thyroid disease can not be stated.

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Acknowledgments

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Addendum

Since this letter was submitted for publication, the following papers have described this procedure, and results, in greater detail: (*a*) Beutner, E. H.; Witebsky, E.; Rose, N. R., and Gerbasi, J. R.: Localization of thyroid and spinal cord autoantibodies by fluorescent antibody technique, *Proc. Soc. Exper. Biol. & Med.* **97**: 712–716, 1958. (*b*) Hiramoto, R.; Engel, K., and Pressman, D.: Tetramethylrhodamine as immunohistochemical fluorescent label in the study of chronic thyroiditis, *Proc. Soc. Exper. Biol. & Med.* **97**: 611–614, 1958.

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A CASE OF MASCULINIZING OVARIAN TUMOR: USE OF CORTICOSTEROID INHIBITION TEST IN DIAGNOSIS

To the Editor:

Virilism in the adult female which is associated with an elevated urinary excretion of 17-ketosteroids but no clinical or laboratory evidence of glucocorticoid excess (Cushing's syndrome) may be due to bilateral adrenal hyperplasia, or to an adrenocortical or ovarian tumor.

A number of reports (1–4) have indicated that the 2 adrenal lesions may be differentiated according to the response of urinary 17-ketosteroid excretion to the administration of corticosteroid. Thus in bilateral adrenal hyperplasia, administration of corticosteroid (*e.g.*, cortisone, prednisone, or 9-alpha-fluorohydrocortisone) will lower the 17-ketosteroid output to well within normal limits. This occurs presumably because the 17-ketosteroid output is under the control of corticotropin (ACTH), and the administration of exogenous corticosteroid inhibits endogenous ACTH secretion and leads to diminished stimulation of the hyperplastic adrenal cortex. On the other hand, adrenal carcinomas are generally independent of control by ACTH, and in these cases inhibition of ACTH secretion will not affect the 17-ketosteroid output.

The 17-ketosteroid response to corticosteroid administration in cases of virilizing ovarian tumor has not been previously reported. One might anticipate a slight fall in the 17-ketosteroid output due to inhibition of the apparently normal adrenal cortex. Increased excretion of 17-ketosteroids might continue, however, due to continued androgen output from the ovarian lesion.

In the present report a patient is described who had virilism and an elevated urinary excretion of 17-ketosteroids, but no abdominal or pelvic mass which could be detected on clinical or radiologic examination. In this patient a diagnosis of masculinizing ovarian tumor was made preoperatively on the basis of the 17-ketosteroid response to administration of prednisone.

Mrs. K. W., aged 38, was admitted to the Toronto General Hospital in April, 1957. Her chief complaints were amenorrhea of one year's duration, and increasing hirsutism (of face, arms and thighs), acne, increased libido and increased irritability—all of six months' duration. She had previously been investigated in another hospital, where a dilation and curettage of the uterus had not shown any abnormality.

On examination she was seen to be a short, obese, rather euphoric woman. No buffalo hump was noted. She had some coarse black hair on her cheeks and chin, which she shaved daily. There were coarse, dark hairs on her arms, over her breasts, and on the lower abdomen and thighs. There was an acneiform eruption over the breasts, upper arms and back. The blood pressure was 160/110 mm. Hg. The abdomen was obese, but no masses were felt.

Pelvic examination revealed no hypertrophy of the clitoris. There was a purulent vaginal discharge due to Trichomonas infection. The uterus was normal in size and no adnexal masses were felt.

The intravenous pyelogram showed nothing abnormal. The urinary 17-hydroxycorticoid excretion averaged 4.3 mg. per twenty-four hours (normal, 1 to 10 mg.). Urinary 17-ketosteroid excretion was slightly elevated, the values ranging from 14.1 to 19.8 mg. per twenty-four hours (normal, 5–14 mg.). The effect of prednisone on urinary 17-ketosteroid excretion is shown in Table 1. It may be seen that the administration of prednisone resulted in a slight fall in 17-ketosteroid excretion, which averaged 3.7 mg. per day. This fall was definitely smaller than had been previously observed in patients

TABLE 1

Day	Therapy	Urinary 17-ketosteroids (mg./24 hrs.)	
1	0	19.8	
2	0	14.9	
3	0	16.2	Average, 16.1 mg.
4	0	15.6	
5	0	14.1	
6	Prednisone 20 mg., orally	12.6	
7	Prednisone 20 mg., orally	13.2	
8	Prednisone 20 mg., orally	12.1	
9	Prednisone 20 mg., orally	15.9	Average, 12.4 mg.
10	Prednisone 20 mg., orally	9.7	
11	Prednisone 20 mg., orally	12.1	
12	Prednisone 10 mg., orally	10.6	
13	Prednisone 7.5 mg., orally	12.2	

with adrenal hyperplasia, and proportionately greater than that seen in patients with adrenal carcinoma. This was the kind of response one might predict in the presence of an ovarian tumor. Hence a provisional diagnosis of masculinizing ovarian tumor was made.

Pelvic laparotomy was performed by Dr. William Allemang. At operation the left ovary was found to be enlarged to twice normal size. It was freely mobile and the capsule appeared perfectly intact and smooth. The left ovary was bisected, whereupon a tumor measuring approximately 3 cm. in diameter was revealed. The left ovary and tube were removed. Histologically, the tumor was an arrhenoblastoma in which there was an unusually high proportion of Leydig cells.

Postoperatively the patient's urinary 17-ketosteroid excretion fell to an average of 6 mg. per twenty-four hours. Her menstrual periods returned within a month after operation and thereafter occurred every two to three weeks. Her voice became somewhat more feminine, hirsutism diminished (especially over the cheeks), and she noticed a marked decrease in the feeling of tension which had previously disturbed her. The blood pressure averaged 135/90 mm. Hg.

From the results of the corticosteroid inhibition test in this patient, it is felt that it *may* be possible to differentiate adrenal tumors from ovarian tumors as a cause of virilism when, as is often the case, they cannot be

localized on physical or radiologic examination. It must be admitted that the fact that the increase in urinary 17-ketosteroid excretion was only moderate in this case made it more easily possible to interpret the results obtained with the prednisone inhibition test. It is apparent that such a test would be of little value if the ovarian tumor secreted a large amount of 17-ketosteroids (*e.g.*, more than 30 mg. per twenty-four hours), and if this amount tended to vary greatly from day to day. In such a case, any small decrease in urinary 17-ketosteroid excretion as a result of inhibition of the normal adrenal cortex would be obscured by the day-to-day variation in secretion from the ovarian lesion. It is also apparent that in the presence of a masculinizing ovarian tumor with normal total 17-ketosteroid output (5), the corticosteroid inhibition test would be of little diagnostic value.

It has been our custom (6) in adult women with virilism who have a high 17-ketosteroid excretion which does not fall to normal levels or below during corticosteroid therapy, to consider that either an adrenocortical or an ovarian tumor is present. In the absence of clinical or radiologic evidence of localization of this tumor, it has been felt that further differentiation of the 2 lesions could not be achieved prior to operation. Generally, the technically more simple operation, pelvic laparotomy, has been performed first. However, the present report indicates that in some of these cases, on the basis of the corticosteroid inhibition test, it may be possible to make a definitive diagnosis preoperatively.

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RAPID RADIOIODINE SUPPRESSION TEST USING TRIIODOTHYRONINE

TO THE EDITOR:

The ingestion of desiccated thyroid or triiodothyronine (T3) inhibits the collection of radioiodine (I^{131}) by the thyroid gland in euthyroid subjects but does not appreciably alter the uptake in hyperthyroid patients (1–3). This difference has been systematized into a clinical test as an adjunct in the diagnosis of hyperthyroidism by Greer and Smith (4), Werner and Spooner (5), and Perlmutter and Slater (6). When triiodothyronine was used, it was administered orally in doses of 35 to 150 micrograms per day for seven to fourteen days and the thyroidal I^{131} uptakes before and after administration of T3 were compared. We have been able to shorten the test by administering 300 micrograms of T3 per day for two days.

METHOD AND PATIENTS

A preliminary determination of the 24-hour thyroidal radioiodine uptake after a 25-microcurie dose of I^{131}, was performed by conventional methods. T3 was then administered orally in a divided daily dosage of 300 micrograms for two days. The I^{131} uptake determination was repeated with appropriate corrections for residual radioactivity.

Thirty-five of the subjects tested were euthyroid. Most of them had borderline or elevated initial thyroidal accumulations of I^{131}, the range being 26 to 65 per cent of the administered dose, with a mean of 48.8 per cent. Nine of these euthyroid subjects had nodular goiters and 7 had slight diffuse thyroid enlargement.

There were 18 patients with hyperthyroidism; 10 had nodular goiters, and 8 had diffuse goiters. In 12 additional normal subjects (euthyroid and without goiter) 2 radioiodine tracer studies were performed, but no T3 was given. In a number of instances the 8-day test described by Werner (5) was performed for comparison.

RESULTS

The mean values for the euthyroid and hyperthyroid subjects are shown in Table 1. The numerical fall in thyroidal I^{131} uptake induced by administration of T3 was 22.7 per cent in the euthyroid group. Twenty-two of the 35 euthyroid subjects showed decreases of 20 per cent or greater, and 27

showed decreases of 15 per cent or greater. All of the final values were within the euthyroid range (*i.e.*, below 50 per cent). Two subjects, however, failed to respond significantly to the inhibitory effect of T3. The first one had an initial uptake of 53 per cent, an uptake of 49 per cent after receiving 300 micrograms of T3 for two days, and an uptake of 56 per cent after receiving 150 micrograms of T3 per day for an additional eight days. She was an elderly woman with a small nodular goiter whose other measures of thyroid function were consistently normal and who has remained clinically euthyroid during two years of observation. The

TABLE 1. THE EFFECT OF TRIIODOTHYRONINE (T3) ON THE THYROIDAL 24-HOUR RADIOIODINE UPTAKE IN 35 EUTHYROID AND IN 18 HYPERTHYROID SUBJECTS

Diagnosis	Thyroidal uptake of I^{131} (%)				
	Before T3 (%)	After T3 (%)	Numerical fall (%)	Per cent change	S.D.
Euthyroid	48.8	26.2	22.7	46.7	8.8
Hyperthyroid	81.1	75.3	5.8	7.0	8.6

second patient had an initial uptake of 55 per cent, which fell only to 46 per cent after the 48-hour suppression test, and was 62 per cent after an additional 8-day test. Though her serum protein-bound iodine level was elevated (8.0 to 10.5 μg. per 100 ml.) and a scintigram revealed a "hot nodule," she has remained clinically euthyroid for a 2½-year period of observation.

In the hyperthyroid group the mean fall in thyroidal I^{131} uptake was 5.83 per cent; the uptake after T3 was below 50 per cent in only 1 instance. This patient had been previously subjected to thyroidectomy for hyperthyroidism and showed recurrence of toxicity.

The difference in response to T3 clearly separated the euthyroid from the hyperthyroid subjects. Using twice the standard deviation of the means, there was no overlap of the final I^{131} uptake values in the 2 groups after administration of T3. A correlation value of 0.92 was obtained (7) for comparison of the 2 tracer tests in the control subjects not given T3. This was significant at the 0.01 level.

DISCUSSION

Following the administration of 300 micrograms of triiodothyronine daily for two days, there was a significant difference in the degree of inhibition of thyroidal I^{131} uptake in euthyroid compared to hyperthyroid patients. Though the magnitude of inhibition was not as great as has been reported with more prolonged tests the values were clinically useful as

an aid in the differential diagnosis of hyperthyroidism. All of the final values in the euthyroid subjects and only 1 final value among the hyperthyroid patients were within the normal range. It seems likely that a more clear-cut differentiation between the 2 groups would have been obtained if we had not included goitrous subjects among the euthyroid group, since it has been shown by Werner and others that some patients with nontoxic nodular goiters do not respond. However, we believed that the euthyroid group should be representative of subjects in whom a diagnosis of hyperthyroidism might be reasonably entertained, in order to subject this test to a more rigorous trial (*i.e.*, subjects with initial high I^{131} uptakes and/or goiters). The T3 radioiodine suppression test is rarely required in subjects whose initial I^{131} uptakes fall well within the normal range and in whom the thyroid gland is normal upon palpation. No toxic effects were observed in any patient receiving 300 micrograms of T3 daily for two days.

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The Endocrine Society

RECIPIENTS OF THE 1958 AWARDS

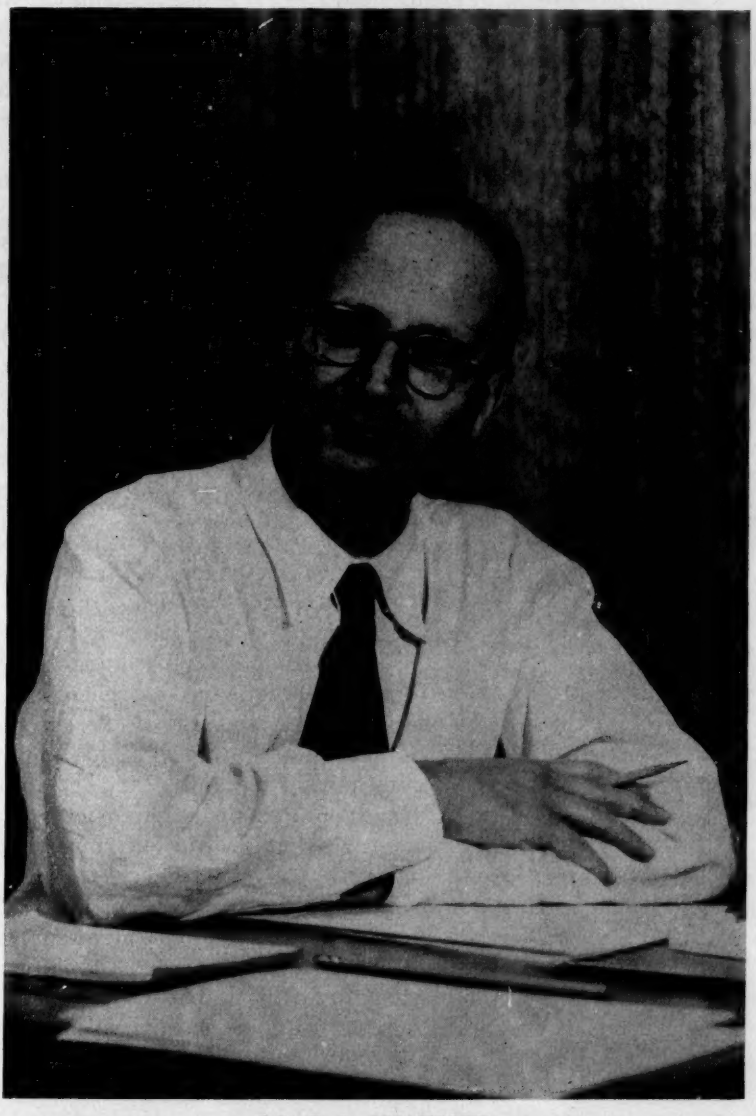
THE MEDAL OF THE ENDOCRINE SOCIETY

Although the scientific contributions of *Dr. I. Lyon Chaikoff* have by no means been restricted to the field of endocrinology, it is therein that this prolific and many faceted investigator has had the greatest influence. He was born in London, England, July 2, 1902. He grew up, however, in Toronto and graduated from the University of Toronto in 1920 at the age of 18. Under the direction of Professor J. J. R. MacLeod he received an M.A. in 1921 and a Ph.D. in 1924. In this period when the work of Banting and his coworkers burst with such impact upon the field of diabetes he carried out pioneering studies, such as that concerned with keto-acid metabolism which antiquated the concept that "fats burn in the fire of carbohydrate." While in the midst of these studies and teaching in the Physiology Department, he paused long enough to acquire an M.D. degree. In 1930 he joined the faculty of the University of California at Berkeley and was appointed Assistant Professor the following year. In 1939 he was named Associate Professor and has been Professor of Physiology since 1942. In 1941 he was awarded a fellowship of the John Simon Guggenheim Foundation.

From his early interest in the metabolic derangements in diabetes and the natural light that they shed upon normal metabolism, his interests have become so broad as almost to defy characterization. From atherosclerosis to zinc metabolism he has pioneered and paved numerous pathways, using the methods of numerous disciplines and the spectrum of biologic material from algae to elephants. It is, indeed, difficult to find an important area wherein he has not ranged during the last two decades: isotope labeling and tracer theory, lipotropic agents, carbohydrate, lipid, protein and mineral metabolism, alcohol, atherosclerosis, radiation-biology, carcinogenesis, nutrition, and metabolic antagonism, to cite only some.

Stemming from his early association with the Toronto investigators has been a continued and productive interest in diabetes mellitus. In 1952 his Harvey Lecture dealt with the metabolic defects in this condition. Although his contributions to our understanding of intermediary lipid, carbohydrate, and protein metabolism in the normal and diabetic would

AWARDED
THE MEDAL OF THE ENDOCRINE SOCIETY



DR. I. L. CHAIKOFF

more than suffice to place him in the first echelon of endocrine investigators, his activities have not been limited to that large area.

His observations of thyroid structure, chemistry, and function have over a span of slightly less than twenty years become major stepping stones in the path toward clarification and delineation in this field. Starting with a systematic investigation of iodine metabolism in the thyroid, using the powerful new tool of radioactive iodine and more sophisticated methods of iodine microchemistry, he has continued to maintain and extend these contributions up to the present time.

In addition to the interest in the thyroid and pancreatic islets, his endocrine endeavors have encompassed cytology, oncology and physiology of the anterior pituitary; the endocrine hypothalamus; lactation; and gonadal and adrenal steroid metabolism and their relation to atherogenesis, fatty liver and cirrhosis. His work has been characterized not only by high quality, fundamental character, and universal acceptance, but also by its wide diversity of area, interest, technique and approach.

The Endocrine Society finds it an honor to recognize such a distinguished investigator.

DR. MONTE A. GREER

THE CIBA AWARD

Dr. Monte Arnold Greer is the recipient of the Ciba Award for 1958 in recognition of ten years of distinguished research. He was born in Portland, Oregon, on October 26, 1922, and after attending Stanford University and its medical school, began a career of investigation at the age of 24. His aptitude and imaginative approach were quickly recognized and led to a sequence of increasingly important appointment in several institutions, including the New England Center Hospital and Tufts University School of Medicine, the Evans Memorial Hospital and Boston University School of Medicine, the National Cancer Institute and George Washington University School of Medicine, the Radiosiotope Service of the Veterans Administration Hospital in Long Beach, California and, finally, in his own home town to the appointment of Associate Professor of Medicine in the University of Oregon Medical School.

The major accomplishments of Dr. Greer have included the determination of the chemical nature of an antithyroid agent in foods, the control

of simple goiter by thyroid administration, and the hypothalamic regulation of anterior pituitary secretion. He has described his carefully conducted experiments on these and related topics in some 50 well-written papers.

The antithyroid action of a large number of foods was surveyed by determining their interference with the accumulation of radioactive iodine by the human thyroid. The potency of the brassica family of plants was noted, and rutabaga was chosen for chemical study. He then participated in the isolation of the antithyroid agent and in its identification as L-5-vinylthiooxazolidone. He has recently isolated and identified the naturally occurring precursor, progoitrine, a glucoside which is enzymatically cyclized to the active oxazolidone when the raw food is crushed. This solution of a long-standing problem contributes importantly to an understanding of the influence of foods on the thyroid gland and of the entire problem of goiter.

Dr. Greer's much-quoted study demonstrating the suppressive effects on thyroid function in normal individuals of orally administered thyroid provided a scientific rationale for the successful use of thyroid to produce regression of nontoxic goiters and thyroid nodules. Additionally, his finding that the thyroid gland of thyrotoxic individuals resisted functional suppression by administration of thyroid provided a useful and widely used diagnostic procedure for thyrotoxicosis.

A clear demonstration of the hypothalamic control of thyrotropin secretion resulted from studies on the effects of hypothalamic lesions, and the location of the controlling area was described. The goitrogenic effects of antithyroid compounds were eliminated by effective lesions, and the center for control of thyrotropin was shown to be different from those concerned with the secretion of corticotropin and gonadotropin and those related to obesity or emaciation. Dr. Greer was also the first to provide evidence for a "drinking center" in the hypothalamus, and he further showed that the state of persistent estrus due to hypothalamic lesions was corrected by chronic administration of progesterone.

Dr. Greer's impressive achievements in endocrine research, and his continuing productivity make him an outstanding recipient of the Ciba Award.

THE SQUIBB FELLOWSHIP

The Squibb Fellowship in Endocrinology for 1958 is awarded to *Dr. Eugene Spaziani.*

Dr. Spaziani received the Bachelor of Arts degree in zoology in August 1952, and the Master of Arts degree in general and endocrine physiology

DR. EUGENE SPAZIANI

in January 1954 from the Department of Zoology, University of California, Los Angeles. He completed his work toward the degree of Doctor of Philosophy at the same institution early in 1958, working with Dr. Clara M. Szego on primary mechanisms of steroid hormone action, with particular reference to permeability alterations in the target organ.

This Fellowship is awarded to Dr. Spaziani in order to permit him to undertake a year of postdoctoral training under the guidance of the distinguished authority on permeability mechanisms, Dr. Hugh Davson at University College, London.

Dr. Spaziani brings to his chosen career in basic endocrinology the broad evolutionary viewpoint of the trained biologist, an acute appreciation of the nature of fundamental problems, the technical capacity for application of the tools of modern biochemistry and physiology to these problems and, above all, complete dedication to the scientific method and to a lifelong career in basic research.

The Awards Committee has every reason to predict that his career will reflect credit upon our Society.

THE SCHERING SCHOLAR

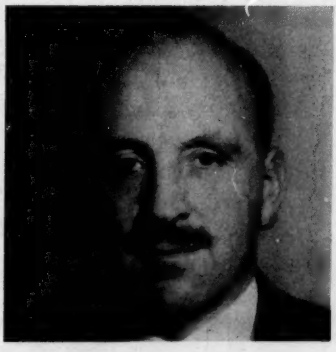
DR. ROY O. GREEP

Dr. Roy Orval Greep, Editor of Endocrinology for the past six years, was born in Longford, Kansas in 1905, giving prominence to a region which was later to be identified also with Abilene. After graduation from Kansas State College he obtained M.A. and Ph.D. degrees from Wisconsin. From 1935 to 1938 he was Assistant and Instructor in Zoology in the Biological Laboratories at Harvard, and after six years of research at the Squibb Institute of Medical Research returned to head research at Harvard's new School of Dental Medicine. Currently he is Professor of Anatomy and Dean of the School of Dental Medicine, and Acting Head of the Department of Anatomy at the Medical School. The broad scope of Dr.

Greep's contributions to endocrinology range over the anterior and posterior pituitary hormones, the hormones bearing upon reproduction, and those of the parathyroids, adrenals and pancreas. He was the first to establish that thyrotropin and luteinizing hormone are separable, that the thyroid can be activated by means other than thyrotropin, that full function can follow transplantation of the anterior pituitary provided the graft is made in the normal position of this gland, and that the adrenal cortex is indeed two anatomically and functionally distinct glands.

Dr. Greep plans to undertake what he describes as "an endocrinologic refueling operation" by visiting universities, laboratories, and European cities (including Paris). The Endocrine Society feels it a privilege to contribute to this praiseworthy undertaking by making him the Schering Scholar for 1958.

THE UPJOHN SCHOLAR



DR. PETER JACKSON

Dr. William Peter Upritchard Jackson has been named the Upjohn Scholar of the Endocrine Society for 1958 to permit him travel in the United States. Dr. Jackson, born in England in 1919, graduated with honors from Cambridge University and received his medical training there and at the London Hospital. Nine years ago he went to South Africa where he now holds the posts of Senior Lecturer in the Department of Medicine and Fellow of the University of Cape Town, Physician and Deputy Head of the Groote Schuur Hospital, Chief of the Endocrine Clinic and Endocrine Research Laboratories, and Assistant Director of the Council of Scientific and Industrial Research. His contributions to clinical medicine and especially to endocrine and metabolic diseases, prediabetes, calcium and phosphorus metabolism, and nutrition, as well as his broad interests in the out-of-doors, insure that his visits here will be profitable both to him and to us.

THE 1959 AWARDS

The selection of the recipients of the awards of The Endocrine Society is made by a Committee appointed by Council of the Society. These awards and fellowships carry no obligation by the recipient to the Society or to the donors.

MEDAL OF THE ENDOCRINE SOCIETY

In 1954 the Council of the Society voted to establish a medal and an honorarium of $1,000 to be given to an individual for work of special distinction in endocrinology. The recipient shall be chosen from nominations presented by members of the Society and is limited to citizens of the United States and Canada. Dr. Carl R. Moore was the recipient in 1955, Dr. Frederick L. Hisaw in 1956, Dr. Joseph C. Aub in 1957, and Dr. I. L. Chaikoff in 1958.

THE CIBA AWARD

The Ciba Award, to recognize the meritorious accomplishments of an investigator not more than 35 years of age in the field of clinical or preclinical endocrinology, was established in 1942, but no recipient was selected in 1942 or 1943. In 1944 the Award was presented to Dr. E. B. Astwood; 1945—Dr. Jane A. Russell; 1946—Dr. Martin M. Hoffman; 1947—Dr. Choh Hao Li; 1948—Dr. Carl Heller; 1949—Dr. George Sayers; 1950—Dr. Oscar M. Hechter; 1951—Dr. Albert Segaloff; 1952—Dr. Seymour Lieberman; 1953—Dr. Sidney Roberts and Dr. Clara Szego (Mrs. Roberts); 1954—Dr. Isadore M. Rosenberg; 1955—Dr. Jack Gross; 1956—Dr. Alfred M. Bongiovanni; 1957—Dr. Nicholas S. Halmi; 1958—Dr. Monte A. Greer. Prior to 1952 the Award was $1,200. It is now $1,800. If within twenty-four months of the date of the award, the recipient should choose to use it toward further study in a laboratory other than that in which he is at present working, it will be increased to $2,500.

THE AYERST AND THE SQUIBB FELLOWSHIPS

The Ayerst Fellowship was established in 1947 and the Squibb Fellowship in 1956. They are designed to assist men or women of exceptional promise in furthering their advancement towards a career in endocrinology. Each Fellowship is awarded on alternate years and the stipend, which will not exceed $5,000, may be divided into two Fellowships in varying amounts in accordance with the qualifications of the appointees. Individuals possessing the M.D. or Ph.D. degree, or candidates for either of these degrees, are eligible for appointment.

Applicants must submit the following information:

1. Evidence of scientific ability as attested by studies completed or in progress.
2. Recommendations from individuals familiar with the candidate and his work.
3. A proposed program of study.
4. Acceptance of the individual by the head of the department in which the Fellowship will be held.

5. A statement that he or she will serve full time if awarded a Fellowship. A small amount of time (10 to 15 per cent) may be spent in course work or participation in teaching, the latter purely on a voluntary basis.

THE SCHERING AND THE UPJOHN SCHOLARS OF THE ENDOCRINE SOCIETY

The Council of The Endocrine Society has established a category of Scholars. These grants have been made available through the generosity of the Schering Corporation and the Upjohn Company, and will be awarded to established investigators and teachers in the field of endocrinology who wish to extend their opportunities for work either in this country or abroad.

The awards will not exceed $2,500 annually for each individual and will be granted on the basis of proposals submitted by the applicant. Such applications should include the estimated financial needs. The funds may be used for travel, maintenance and other expenses.

Nominations

Nominations for the Medal of the Endocrine Society; the Ciba Award; and the Ayerst and the Squibb Fellowships may be made by any member of The Endocrine Society. They should be submitted on forms which may be obtained from the Office of the Secretary, 1200 North Walker Street, Oklahoma City 3, Oklahoma. Completed nominations should be returned to the Secretary not later than *November 1* each year.

Proposals for appointments as Scholars of The Endocrine Society should be made in writing by the individual, and addressed to the Secretary of the Society. They should be submitted by *November 1* each year.

The Awards Committee will meet in November and notice of awards to successful nominees and applicants will be made not later than December 1.

TENTH POSTGRADUATE ASSEMBLY IN ENDOCRINOLOGY AND METABOLISM

SPONSORED JOINTLY BY THE ENDOCRINE SOCIETY AND THE STATE UNIVERSITY OF NEW YORK, UPSTATE MEDICAL SCHOOL

SYRACUSE, N. Y.

September 29 through October 3, 1958

For information concerning the program and registration, write to *Charles W. Lloyd, M. D., State University of New York, Upstate Medical Center, Syracuse 10, N. Y.*

The registration fee is $100.00. Residents and Fellows will be admitted for a reduced fee of $30.00. Those wishing to reserve rooms should communicate directly with the Hotel Syracuse.

PROGRAM

Monday (A.M.)	**September 29, 1958**
8:00– 9:00	Registration: Lobby of the State University College of Medicine
	Introduction to Endocrinology and the Endocrine Patient
9:00–10:00	General Concepts of Endocrinology—Dr. Jay Tepperman
10:00–10:30	Examination of the Endocrine Patient—Dr. Roberto Escamilla
10:30–12:00	Use of the Endocrine Laboratory; Exhibits—The Faculty
12:00–12:30	Question Period
Monday (P.M.)	*The Endocrine Patient—Psychology Aspects:*
	Panel Discussion with Case Presentations
2:00– 5:30	The Effects of Various Endocrine States on Behavior and Cerebral Function.
	The Impact of Altered Emotional States on Endocrine Function.
	Drs. G. Engel (moderator), Ham, Hamburg, Hollender and F. Engel (One member of this panel will be present at each subsequent session to discuss any pertinent psychological aspect.)
6:30	Get acquainted cocktail party.
Tuesday (A.M.)	**September 30, 1958**
9:00–12:00	*Panel Discussion with Case Presentations:*
	Disorders of the Hypothalamus and Pituitary Gland
	The Hypothalamico-Hypophyseal Axis, Hypothalamic Syndromes including Diabetes Insipidus, Clinical Syndromes and Management of Hypo and Hyperfunctions of the Adenohypophysis, Surgical Aspects of Pituitary Tumors.
	Drs. Escamilla (moderator), Albert, King and Lloyd.
12:00–12:30	Question Period
Tuesday (P.M.)	*Panel Discussion with Case Presentations:*
	Disorders of the Thyroid Gland

2:00– 5:30 Anatomical and Physiological Considerations, Hyperthyroidism, Hypothyroidism, Colloid goiter, Toxic and Non-toxic Adenomata, Thyroid cancer, Thyroiditis, Exophthalmos, Use and Abuse of Thyroid Hormone.
Drs. Rawson (moderator), Dobyns, Gardner, Rall and Turner.

Wednesday (A.M.) **October 1, 1958**
Panel Discussion with Case Presentations:
Disorders of the Adrenal Cortex
Physiological and Biochemical Considerations including the Actions of the Newer Steroid Analogs, Spontaneous and Induced Adrenal Insufficiency, Aldosteronism and Abnormalities of Electrolyte and Water Metabolism.
Drs. F. Engel (moderator), Conn, Forsham and Henneman.
12:00–12:30 Question Period
Wednesday (P.M.) *Panel Discussion with Case Presentations:*
2:00– 5:00 *Disorders of the Adrenal Cortex and Medulla*
Hyperadrenocorticism including Cushing's Syndrome and Adrenal Virilizing Syndromes in Children and Adults, Pheochromocytoma.
Drs. Forsham (moderator), Conn, F. Engel and Gardner.
5:00– 5:30 Question Period

Wed. evening Cocktail hour and dinner.

Thursday A.M.) **October 2, 1958**
9:00–10:00 *Lecture:* Embryogenesis of the Gonad, Physiology of the Ovary and Testis—Dr. Nelson
10:00–12:30 *Panel Discussion with Case Presentations:*
Disorders of the Male Gonad
Syndromes of Hypogonadism, Male Infertility, Use of Androgen, Sexual Precocity in Males, Steroid Secreting Tumors of the Testis.
Drs. Nelson (moderator), Albert, McCullagh and Simmons.
Thursday (P.M.) *Panel Discussion with Case Presentations:*
2:00– 5:00 *Disorders of the Female Gonad*
Ovarian Hypofunction Syndromes Including Menopause and Amenorrhea, Ovarian Dysfunction Syndromes including Functional Uterine Bleeding and Premenstrual Distress, Female Infertility, Steroid Secreting Tumors of the Ovary, Sexual Precocity in Females.
Drs. Greenblatt (moderator), Lloyd, Simmons and Sturgis.
5:00– 5:30 Question Period

Friday (A.M.) **October 3, 1958**
9:00–12:00 *Panel Discussion with Case Presentations:*
Metabolic Bone Disease
Physiology of Bone and the Parathyroid Glands, Hyper and Hypoparathyroidism, Osteoporosis, Osteomalacia.
Drs. F. Engel (moderator), Henneman, Neuman and Ray.
12:00–12:30 Question Period
Friday (P.M.) *Panel Discussion with Case Presentations:*
2:00– 4:00 *Oncology and Endocrinology*
Management of Patients with Cancer of the Breast and of the

Prostate, Tumors of Endocrine Origin including Chorioepithelioma.
Drs. Pearson (moderator), Baker, Bergenstal and Mueller.

4:15– 5:30 Question Period and Summary of the Course

ACKNOWLEDGMENTS

The Editorial Board of THE JOURNAL OF CLINICAL ENDOCRINOLOGY AND METABOLISM wishes to acknowledge with gratitude the generous and excellent help of the following reviewers:

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THE GERONTOLOGICAL SOCIETY, INC.
NOTICE OF MEETING

The eleventh annual scientific meeting of the Gerontological Society, Inc., will be held at the Bellevue Stratford Hotel, Philadelphia, Pennsylvania, November 6, 7, and 8, 1958.

Co-Chairman in Charge of Arrangements, Dr. Joseph T. Freeman, 1530 Locust St., Philadelphia 2, Pa.

POST-GRADUATE COURSE IN GYNECOLOGIC ENDOCRINOLOGY

New York University Post-graduate Medical School announces the following postgraduate course in Gynecologic Endocrinology: A practical, didactic and clinical presentation with emphasis on therapeutic management of endocrine disorders in the female. Endocrine therapy for menstrual abnormalities and ovulatory defects are discussed. Adrenocortical, thyroidal, ovarian and pituitary abnormalities are emphasized. Practical diagnostic tests are discussed and demonstrated.

Five-day, full-time course, *October 13 through 17, 1958*. Tuition, $125.00. Given under the direction of Dr. Herbert S. Kupperman.

For further information, write to The Office of the Associate Dean, NYU Post-graduate Medical School, 550 First Avenue, New York 16, N. Y.

ACADEMY OF PSYCHOSOMATIC MEDICINE—ANNUAL MEETING

The fifth Annual Meeting of The Academy of Psychosomatic Medicine will be held *October 9–11, 1958*, at the Park Sheraton Hotel in New York City. The program will be devoted to "The Psychosomatic Aspects of Internal Medicine" and will include formal papers, panel discussions and luncheon conferences. The meeting will be open to all scientific disciplines, as well as psychologists, social workers and nurses. Information may be obtained from Dr. Bertram B. Moss, Suite 1035, 55 East Washington Street, Chicago 2, Illinois.

BOOK REGISTER

Clinical Enzymology edited by GUSTAV J. MARTIN, Sc.D., Research Director, The National Drug Company, Philadelphia, Pa. In the Introduction, the Editor states:" An effort will be made to outline the basic knowledge essential to an understanding of the field, to review the biochemical basis, to survey the current status of clinical observations, and finally to offer a concept essential to the extension of efforts in this immediate sphere of medical endeavor. No consideration is given to the topical application of enzymes as this has been completely reviewed in other volumes of recent date." There are 7 articles on: Protein Biology; Chemistry of Enzymes Used Clinically; Biochemistry of Enzymes Used Clinically; Parenteral Use of Enzymes in Medicine; Diagnostic Use of Enzymes; Polymerases in Biology; and Recapitulation and Perspectives for Clinical Enzymology; 241 pages; illustrated; 1958. Little, Brown and Company, Boston 6, Massachusetts. Price $6.00.

Hormonii Steroizi. By A. LUPULESCU, M.D., Chief Assistant, Institute of Endocrinology, Bucharest, Roumania. The various chapters of this book cover the following: A General View of the Steroid Hormones; the Biogenesis of Steroid Hormones; Relationship between Hypophysis and Adrenal Cortex in Normal Subjects, Cushing's Syndrome, the Adrenogenital Syndrome and in Tumors; Steroid Hormone Metabolism Studied with the Aid of Radioactive Hormones; Steroid Hormones in Therapy; Aldosterone; Estrogens; Progesterone; Androgens; The Liver and the Intermediate Metabolism of Steroid Hormones; The Dosage of Steroid Hormones and Their Derivatives; Connective Tissue and Steroid Hormones; Neoplasms and Steroid Hormones; Hormonal Treatment of Tumors; and Steroid Homeostasis in the Organism; 400 pages; 146 figures; 1958. Editura Medicală, Bucharest, Roumania.

Modern Trends in Endocrinology. Edited by H. GARDINER-HILL, M.D., F.R.C.P., Consultant Physician to St. Thomas's Hospital, London, England. This book covers research in endocrinology during the past ten years. Twenty-four expert contributors present articles in which the modern trends are appraised and correlated with current practice. These articles are entitled: Present Knowledge of the Thyroid Hormones; Antithyroid Drugs; Aetiology and Treatment of Exophthalmos and Exophthalmic Ophthalmoplegia; Tests of Thyroid Function; Treatment of Toxic and Malignant Thyroid Disorders with Radiocative Iodine; Endocrine Factors in the Syndrome of Diabetes Mellitus; Endocrine Aspects of Overnutrition and Undernutrition; The Pituitary Antidiuretic Hormone (ADH) and the Control of Fluid Balance; New Adrenal Cortical Steroids—Aldosterone and Synthetic Compounds; Role of Adrenocortical and Pituitary Hormones in Water and Electrolyte Control; Adrenal Cortical and Other Factors in Hypopituitary Coma; The Hormones of the Sympathetic Nervous System and of the Adrenal Medulla; Adrenal Function Tests; Cortisone and Corticotrophin in the Treatment of Rheumatic Disorders; Corticotrophin and Cortisone in the Suppression of Allergic and Pararheumatic Disorders; Hormonal Factors in Breast Development and Milk Secretion; Endocrine Factors in the Aetiology and Treatment of Cancer of the Breast and Prostate; Endocrine Treatment of Gynaecological Disorders; The Role of Endocrine Factors in Infertility in the Female; Carcinoid Tumors and Serotonin; and Growth and Development of the Stress Concept and Its Significance in Clinical Medicine; 311 pages; 15 figures; 1 color plate; 1958. Paul B. Hoeber, Inc., New York. Price $13.50.